FACULTY OF VETERINARY MEDICINE
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Development and validation of high-resolution mass spectrometry-based approaches for active and passive sampling of emerging organic micropollutants in the marine environment

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A dissertation submitted to Ghent University in partial fulfilment of the requirements for the degree of Doctor (PhD) in Veterinary Sciences

Academic year: 2018 – 2019

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This work fits within the NewSTHEPS project (BR/143/A2/NEWSTHEPS), supported by the Belgian Science Policy (BELSPO). This doctoral work was executed at the Laboratory of Chemical Analysis (Faculty of Veterinary Medicine, Ghent University).

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Notation index

A

a.u.	arbitrary units
AGC	automatic gain control
ANOVA	analysis of variance
APCI	atmospheric pressure chemical ionization
AP	alkylphenol
APPI	atmospheric pressure photoionization

B

BBP	benzylbutyl phthalate
BPA	Bisphenol A
BPNS	Belgian Part of the North Sea

C

CE	collision energy
CEC	contaminants of emerging concern
CCF	central composited faced-centered design
CID	collision-induced dissociation
$C_{s,t}$	concentration measured in the sorbent phase
$C_{w,t}$	concentration measured in the water phase
CWA	Clean Water Act

D

DAD	diode array detection
DBP	dibutyl phthalate
DEHP	di-ethyl hexyl phthalate
DEP	di-ethyl phthalate
DLLME	dispersive liquid-liquid microextraction
DSPE	dispersive solid-phase extraction
DOP	di-octyl phthalate
DVB	divinylbenzene

E

EDC	endocrine disrupting compound
EE-SPME	electrochemically-enhanced solid-phase microextraction
EPA	environmental protection agency
ESI	electrospray ionization
EQS	environmental quality standards

F

FD	field desorption
FTIR	fourier transform infrared
FWHM	full width at half maximum

G

$\Delta G_{288,15K}$	gibbs free energy at 288.15K
GC	gas chromatography

H

ΔH	enthalpy
HF-LPME	hollow fiber liquid phase microextraction
HPLC	high-performance liquid chromatography
HO	harbour Oostende
HZ	harbour Zeebrugge
HRMS	high-resolution mass spectrometry
H_y	hydrophilic factor

K

K_{sw}	sorbent-water partitioning coefficients

L

LC	liquid chromatography
LDPE	low-density polyethylene
LLE	liquid-liquid extraction
LPME	liquid-phase microextraction

M

MDL	method detection limit

MEC	measured environmental concentration
MESCO	membrane-enclosed sorptive coating
MMLLE	microporous membrane liquid-liquid extraction
MQL	method quantification limit
MRM	multiple reaction monitoring
MS	mass spectrometry
MS/MS	tandem mass spectrometry
MSPE	magnetic solid-phase extraction
MTBE	methyl tert-butyl ether
MW	molecular weight

N

NIR	Near-infrared

O

OO	open sea Oostende
OSPAR	marine convention for the protection from Oslo to Paris
OZ	open sea Zeebrugge

P

PAE	phthalate/phthalic acid ester
PAH	polycyclic aromatic hydrocarbon
PC	polycarbonate
PCB	polychlorinated biphenyl
PDMS	polydimethylsiloxane
PED	polyethylene device
PES	polyethersulfone
PET	polyethylene tetraphthalate
POM	polyoxymethylene
POCIS	polar organic chemical interactive sampler
pKa	logarithm of acid dissociation constant
PNEC	predicted no effect concentration
PRM	parallel reaction monitoring
PRC	performance reference compound
PVC	polyvinyl chloride
PVP	poly-N-vinylpyrrolidone

R

RSD	relative standard deviation
RSM	response surface modelling
RQ	risk quotient

S

ΔS	entropy
SBSE	stir bar sorptive extraction
SDB-RPS	styrene divinylbenzene - reversed phase sulfonated
SDB-XC	styrene divinylbenzene - exchange
SIM	selected ion monitoring
SLE	solid-liquid extraction
SPE	solid-phase extraction
SPME	solid-phase microextraction
SRM	selected reaction monitoring

T

TOF	time-of-flight

U

UHPLC	ultra-high performance liquid chromatography
USAEME	ultrasound-assisted emulsification-microextraction
USE	ultrasonic solvent extraction

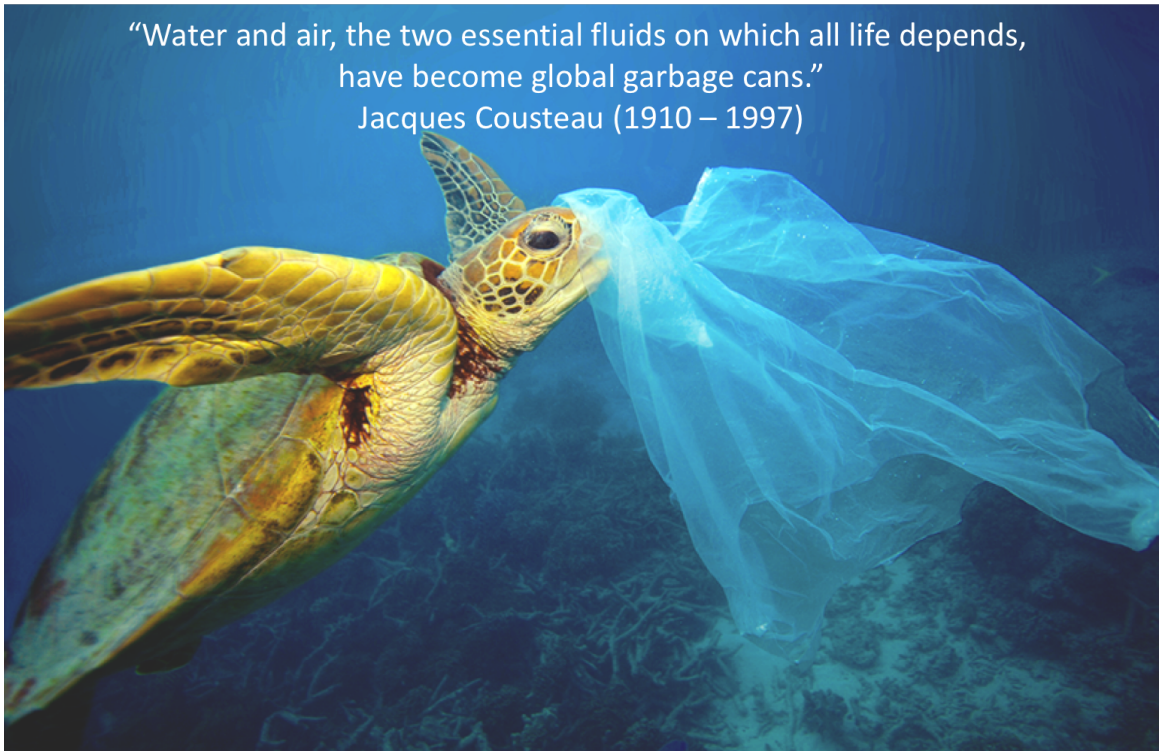
V

Vx	molecular volume

W

WFD	Water Framework Directive
WWTP	wastewater treatment plant

General introduction



1. INTRODUCTION

Water is an essential (re)source, crucial for all living organisms and numerous human activities [1]. Proper functioning of human activities and systems, including food and other industries, financial activities, agriculture, etc. requires a healthy and secure water supply [2]. There are however significant anthropogenic pressures on this delicate (re)source. Indeed, more than 30 % of the global fresh water systems is affected by anthropogenic activities within a domestic, agricultural and/or industrial context [3]. These anthropogenic activities are a consequence of the development of our society and life. Currently, over 100 000 chemical substances are marketed in Europe, and the registration and consumption of chemicals are still increasing [4]. As a result, natural and anthropogenic substances are continuously entering our aquatic environment. The latter include, but not limited to, pesticides, pharmaceuticals, personal care products, plasticizers and hormones. Most of these compounds are at present unregulated in the water supply context and are therefore considered as 'contaminants of emerging concern' or CECs. Compounds that can potentially interfere with the hormonal system of organisms are also designated as 'Endocrine Disrupting Compounds' or EDCs.

Over the past few past decades, the concept of EDCs has risen from total obscurity to nearly a household term [5]. In the beginning of the 1940s, ecologists noticed the first abnormal reproductive patterns in wildlife studies. In 1962 the book Silent Spring by Rachel Carson was essential in awakening both the scientific community as well as the public to the idea that the manufactured chemicals, used in our human civilization can cause harm to ecosystems and human health. A few years later, the government of the United States founded the National Institute of Environmental Health Sciences (NIEHS) (1966) to study how the environment affects human health, while the U.S. Environmental Protection Agency (EPA) (1970) started to implement regulations to protect the environment and human health. During 1960 to 1970, ecologists began to notice unexpected patterns in animals. For example, in the Great Lakes, domesticated mink virtually stopped producing pups [6]. In the early 1970s, the artificial

estrogen diethylstilbestrol (DES) that was prescribed from 1940 to 1971 to millions of women during pregnancy to reduce miscarriage, was identified as transplacental carcinogen. The discovery of DES's tragic legacy was the first time doctors and scientists realized the potential for chemicals to cause physical deformities and more subtle health effects which emerged many years later in life [7]. In 1972, the U.S. EPA announced that the pesticide dichloro-diphenyl-trichloroethane (DDT) would be prohibited in the United States. At the same time, different researchers raised concerns about environmental contamination from oral contraceptives. From then on, the NIEHS held different "Estrogens in the Environment meetings". In 1979 the first one was held in order to frame the larger picture of hormone-mimics and their health effects. The meeting focussed on identifying the properties and diversity of environmental estrogens [8]. The outcome of this meeting was an ecosystem model of R. L. Metcalf that could test the environmental effects of several chemicals, currently recognised as EDCs. Metcalf's research demonstrated how chemicals bioaccumulate and biodegrade in living organisms [8]. The second "Estrogens in the Environment" meeting in 1985s emphasized the effect of environmental estrogens on puberty in young children. Moreover, the meeting examined the biological actions of estrogen exposure, such as reduced sperm counts, testicular cancer, and other negative conditions [9]. As a result of the growing awareness of harmful environmental chemicals, abnormal ecological patterns, and increasing focus on hormone-like chemicals, scientists from a variety of fields convened at the Wingspread Conference in 1991. The latter proved to be a key turning point in the development of the field of endocrine disruption. Since then, the terms "endocrine disruption" and "endocrine disruptors" were used. This meeting was the inspiration and framework for the remarkable research that followed from the 1990s till the early 2000s. For example, studies on turtles showed that PCBs, similar to estrogens, can influence the sex of the offspring [10,11]. With the development of *in vitro* screenings [12], the list of EDCs increased rapidly from a few pesticides (e.g. chlordecone, methoxychlor, and DDT) and industrial chemicals (e.g. PCB), to a variety of new compounds with estrogenic activity that were found in plastics [13,14], disinfectants, and personal care products [15]. Studies began to suggest that EDCs

could cause health effects even at low doses (e.g. growth), whereas opposite effects were observed at high doses [16,17]. As the evidence accumulated on the large picture of EDCs in the environment, the third “Estrogens in the Environment meeting” was held in 1994 to explore the effects in wildlife and draw linkages between estrogen exposures and human diseases [18]. Since 1996, the EDCs have been considered as a global priority. In Weybridge (United Kingdom) “the Impact of Endocrine Disruptors on Human Health and Wildlife” was discussed the international level by regulatory agencies, national authorities and international agencies [5]. In 1999, the European Commission adopted the community strategy for endocrine disruptors, which included short, medium and long-term actions. Calls were launched for more effective ways to identify EDCs, determine levels of exposure, and keep new EDCs from the marketplace. In 2001 (Stockholm convention), persistent organic pollutants were recognised as compounds that can cause serious health effects. The World Health Organization outlined in 2002 the state of the science on endocrine disruption, discussing the mechanisms of action and health effect in animals and humans [19]. In 2009, the Endocrine Society released its first scientific statement on EDCs, presenting evidence-based perspectives and identifying areas requiring additional research [20], which proved to be a milestone in lending legitimacy to the EDC field. Recent consensus and reviews, i.e. report of WHO in 2012 and a 2013 statement by European Food Safety Authority, reinforced these statements [21]. In 2015, the Endocrine Society issued that the policymakers in the European Union needed to define criteria for recognizing EDCs. As a result, in 2016 the European Commission proposed criteria to identify EDCs [22]. In parallel with these regulatory developments and further evolving research, the term EDCs has been acknowledged in his broadest meaning as a compound that can interfere with the function of hormones. An environmental EDC, according to the U.S. EPA, is defined as an exogenous agent that interferes with the synthesis, secretion, transport, binding, action, or elimination of natural hormones in the body that are responsible for maintenance of homeostasis, reproduction, development, and/or behaviour [23]. Currently, the U.S. EPA estimates that about 10 000 unique chemicals have a potential endocrine disrupting activity. Aquatic organisms are particularly susceptible to EDCs, because their entire life cycles are

spent in continuous contact with water [24]. Therefore, the main focus of this work lies on the development and application of monitoring strategies for environmental micropollutants in aquatic matrices, and especially in seawater, that can potentially interfere with the endocrine hormonal system, hereafter thus referred to as EDCs.

2. ENDOCRINE DISRUPTING COMPOUNDS (EDCS) IN THE AQUATIC ENVIRONMENT

2.1. Classification

2.1.1. Steroid hormones (steroidal EDCs)

Steroid hormones are extensively used both in human and veterinary medicine for 3 predominant purposes. First, there is the treatment of diseased living organisms, in which corticosteroids play the most important role. Second, steroid hormones are massively used for controlling the reproductive cycle and synchronizing oestrus in females. Third, steroid hormones can be used albeit illegal as growth promotors in athletes, for meat production purposes and/or for enhancing meat quality. Steroid hormones are considered as most critical pharmaceuticals posing a risk to the environment, because they are continuously and increasingly being used in human and veterinary medicine [25,26]. Furthermore, steroid hormones are recognised as the most potent endocrine disrupting compounds (EDCs), due to the fact that they can interact at very low concentrations (near 1 ng L^{-1}) with the hormonal system of living organisms [27–32]. Interfering steroidal EDCs can have both a natural or synthetic origin, but both natural or synthetic analogues can elicit potential negative effects on the endocrine system of humans and wildlife.

The steroidal EDCs, encompassing both natural and synthetic compounds and their metabolites, can be sub-classified in the androgens, oestrogens, progestins and corticosteroids [33,34]. The androgens, representing the male sex steroids, play a key role in reproduction, sexual maturation and differentiation in males. Additionally, androgens generally

impact normal human development and physiology [35]. Androgens may also exert anabolic effects including an increase of skeletal muscle mass and strength, whereof the mechanisms are not yet fully understood [36]. Synthetic androgens are designed towards more selective anabolic properties, higher oral bioavailability, optimised pharmacokinetics and/or minimized estrogenic side effects [35]. Oestrogens on their hand bring about feminine characteristics, controlling the reproductive cycle and pregnancy but may also influence bone, skin, the cardiovascular system, and immunity [37,38]. Synthetic oestrogens are generally used as ingredients in oral contraceptives. Natural and synthetic oestrogens are suspected to have adverse effects on the endocrine system in wildlife [39] and humans [40]. Consequently, several studies report an increase in oestrogen-dependent diseases, such as testicular, breast, prostate, ovarian and corpus uteri cancers [41,42]. Synthetic progestins, such as medroxyprogesterone acetate, norethindrone and norgestrel, are frequently used in contraceptive treatments for the promotion of the menstrual cycle, symptom control in menopause, and prevention of certain cancer types. Synthetic progestins are often associated with oestrogens in contraceptive treatments [43]. Furthermore, progestins control oestrogen-induced endometrial hyperplasia [44]. Derivatives of progestins, produced through formulation (synthetic production) or obtained following extraction of living organisms, such as the teleost fish, African cichlid, Atlantic cod, Atlantic salmon, eel, goldfish, Japanese medaka, zebrafish and fathead minnow, can show androgenic activity. As a result, they can interact with other steroid receptors, such as oestrogen, progestin, androgen, glucocorticoid and the peroxisome proliferative-activated receptors, of living organisms [45–47]. As a last sub-class of the steroidal EDCS, corticosteroids are anti-inflammatory and immunosuppressant substances, with therapeutic usage in a wide range of disorders, such as asthma, rheumatoid disease, gastrointestinal, cardiac and skin disorders [48].

Generally, steroidal EDCs are excreted by humans and livestock in their native form, as conjugates or as specific metabolites [49,50]. EDC metabolites and/or degradation products are of equal importance as the parent compounds, because they may behave as antagonists and/or agonists of the oestrogen and/or progestagen receptors in hormonal systems [43,51].

The chemical structure of each sub-group of steroidal EDCs studied in this doctoral thesis is presented in Figure 1.

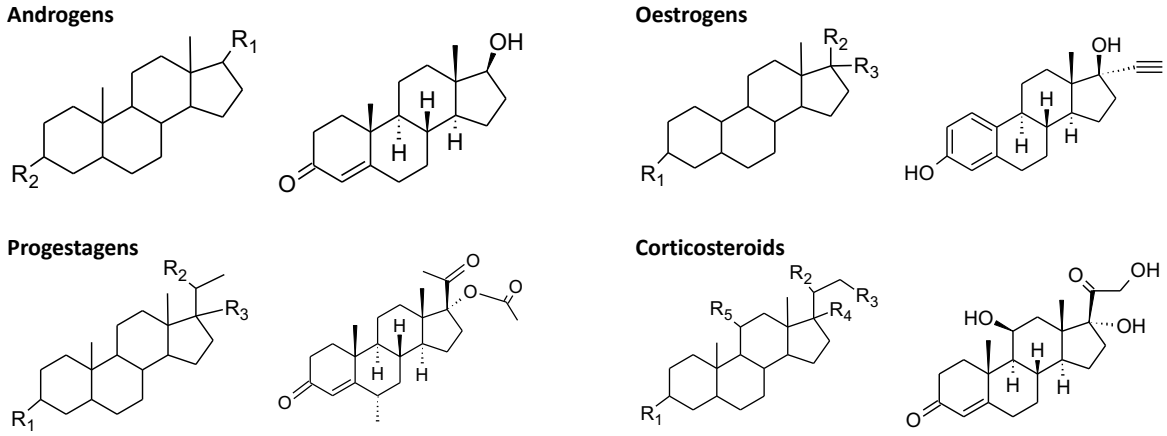


Figure 1. The chemical backbone of the different steroidal EDC classes studied in this doctoral thesis. For the androgens, oestrogens, progestogens and corticosteroids respectively, β-testosterone, 17α-ethinylestradiol, medroxyprogesteronacetate and cortisol are depicted. R-groups represent varying functional groups or side chains.

2.1.2. Plasticizers and plastics additives (man-made EDCs)

Plasticizers and plastics additives are compounds that are used to alter the physical properties of plastics in a number of applications, such as packaging, epoxy resins (coatings), building materials, children's toys, thermal printing paper, medical devices, electronics, plastic food containers, drinking bottles, eye glass lenses and cosmetics [52]. Commonly used plasticizers and plastics additives comprise the phthalates and bisphenol A (BPA) [53,54].

Phthalates are the most used plasticizers with as main application the production of polyvinyl chloride (PVC) plastics and other non-PVC applications (as mentioned before). In particular di-ethyl hexyl phthalate (DEHP) is an exceedingly and widely used one [55]. Phthalates can easily migrate from products, during production or use, to the environment, because they are not bound covalently and are thus easily lost by a variety of physical processes. Furthermore, phthalates are also excreted by humans as a result of direct or indirect contact via food, water, dust and consumer products [56,57]. In the human body, phthalates are rapidly metabolised through phase I reactions (hydrolysis and subsequent oxidation), followed by phase II metabolism and excretion to urine as mono-phthalate esters or conjugates [58]. The low-

molecular phthalates are metabolised into stable hydrolytic mono-esters (primary metabolites). On the other hand, the high-molecular phthalates are metabolised into intermediate hydrolytic mono-esters, which are in turn extensively transformed by ω-, (ω − 1)- and β-oxidation into oxidative secondary metabolites (i.e. alcohols, ketones and carboxylic acids) [59]. Different researchers report that these mono-esters (primary metabolites of phthalates) and the secondary oxidation metabolites possess a similar biological activity as the parent phthalates [60–62]. On top of that, detected traces of parent phthalates have also been used a biomarkers for investigating the exposure of humans [63–67] and aquatic organisms [68] to phthalates.

BPA, characterized by two hydroxyphenyl functionalities, is one of the most highly produced bisphenol analogues with over 3 million tons per year [69,70]. The use of BPA in different polymer applications, may lead to migration of BPA from polymers into food sources (i.e. food and water), and non-food sources (e.g. dust, thermal paper, dental materials, and medical devices). Additionally, BPA is frequently used as metal coating to prevent rusting and corrosion [71,72]. BPA can leach into liquids through two different processes. First, because of its incomplete polymerization during plastic and coating production, residues can diffuse into foods and liquids, particularly at higher temperatures [71,73,74]. Second, hydrolysis of the polymer catalysed by hydroxide ions (OH^-) in aqueous solutions can onset leaching [75], leading BPA to end up in lakes or rivers, where it can be accumulated by aquatic organisms. Furthermore, exposure to BPA has also been demonstrated in humans following the consumption of BPA-based polymer canned food or the food itself [76]. BPA residues have been reported in fruits and vegetables, seafood, meat, eggs, milk, honey, and drinking water [77]. The predominant sources of exposure to BPA of humans are therefore food and water [78]. BPA can also be metabolised by aquatic organisms and humans. BPA is not extensively transformed by phase I reactions, but rapidly conjugated with glucuronic acid (phase II metabolism) to the non-active BPA-glucuronide and to a lesser extent to BPA-sulphate and BPA-chlorate. The formation of BPA conjugates is considered as a detoxification, as only the native BPA forms demonstrate estrogenic activity [79,80]. However, a recent study found that

conjugated forms of BPA can perturb cellular responses in prolactemia cells [81]. However, BPA conjugates can be converted to free BPA by bacterial populations (in WWTPs) through the presence of β-glucuronidase and arylsulfatase enzymes [83,84]. Even more, presence of β-glucuronidase in lysome and endoplasmatic reticulum membranes (in the human liver and the kidney) can reverse detoxification [82,83]. Several studies have demonstrated the presence of BPA in human biofluids, such as urine, blood, and amniotic fluid [78,84,85]. BPA, measured in urine and wastewater treatment plants (WWTPs), has been considered as a reliable biomarker for predicting the overall exposure of humans during assessment studies [85,86]. The chemical structures of some well-known plasticizers and plastics additives are shown in Figure 2.

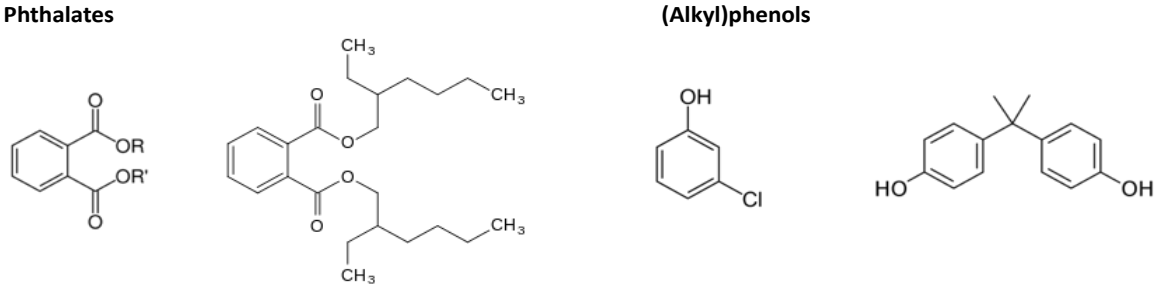


Figure 2. The chemical backbone of phthalates and (alkyl)phenols as studied in this doctoral thesis. For the phthalates, di-ethylhexyl phthalate and for the (alkyl)phenols, 3-trichlorophenol and bisphenol A are shown. The presence of one or two R-group(s) depicts the mono- and di-phthalates, respectively.

2.2. Environmental concentrations and fate

Figure 3 depicts a summary of the quantitative data published so far on the most frequently detected steroidal EDCs, plasticizers and plastics additives in the aquatic environment. This literature survey indicates that the occurrence of the above-mentioned compounds (depicted in Figure 1 and Figure 2) has been mainly surveyed in fresh water environments, i.e. surface water (such as lakes and rivers), ground water, drinking water and wastewater. Steroidal EDCs are generally reported in lower concentrations (< 0.1 up to 100 ng L^{-1}) as compared to plasticizers and plastics additives in fresh water environments (10 up to 1000 ng L^{-1}). For the plasticizers and plastics additives higher concentrations were observed in wastewater and

even in marine waters, although at present only a limited amount of data is available for those matrices.

In surface water, almost all steroidal EDCs, plasticizers and plastics additives have been detected (except for norgestrel and di-isonyl phthalate). It is therefore not surprising that 65% of the rivers, including important aquatic habitats, are currently classified as moderately to highly threatened [87]. In ground and drinking water, several studies on the presence of steroidal EDCs [88,89], plasticizers and plastics additives [89–91] have been published so far. Furthermore, the detected range of micropollutant concentrations in raw and treated wastewater is higher than in surface waters. Although WWTPs offer a major shackle between industrial activities and the environment, still quantifiable levels of steroidal EDCs have been detected in WWTP effluents [92–100]. Evidently, residual steroidal EDC concentrations can leach to other parts of the aquatic environment. Although conjugates of steroidal EDCs have been recognized to exert lower biological activities than their native forms, cleavage to free steroidal EDCs can occur through bacterial populations (in WWTPs) that are capable of producing β-glucuronidase and arylsulfatase enzymes [101,102]. Indeed, the prevalence of free steroidal EDCs in treated wastewater and rivers offers an indication that metabolites can be converted back into their active form [97,101,103–106]. With respect to the plasticizers and plastics additives (except for BPA), almost none of them have been monitored towards their removal from wastewater [95,107–110]. It should be noted that di-n-butyl and di-iso-butyl phthalate were not simultaneously detected in wastewater and marine water, although both were successfully chromatographically separated and analysed with similar detection limits of the applied analytical method [69]. Figure 3 demonstrates that, based on the limited data available on animal farm wastewater, the use of steroidal EDCs in animal farming activities (beef cattle and swine production) also contributes to the contamination of our wastewater (detected concentrations up to 1000 ng L^{-1}) [112,113]. Moreover, similar and or even higher concentrations were detected in animal farm wastewater in contrast to wastewater originating from WWTPs. To the best of the authors' knowledge, no comparable quantitative information is available for the intake of steroidal EDCs by humans and livestock. Nevertheless, a recent

study of Adeel et al. (2017) highlighted that the estrogen discharge by livestock is more than twice the rate of human discharge [114]. Globally, approximately 21 ton/year of oestrogens are discharged into the environment [114].

The most frequently reported steroidal EDCs, plasticizers and plastics additives are the oestrogens and di-phthalates, whereas data for androgens, progestogens, corticosteroids and mono-phthalates are more limited. Scarcity of monitoring data is certainly an issue when it comes to the marine environment. Moreover, studies focusing on the fresh water environment, primarily map the local anthropogenic contamination. To obtain a better picture on the general contamination status, the marine environment should be investigated as well. This was also reinforced by the work of Noppe et al (2007) [115]. The latter examined a limited number of steroidal EDCs (i.e. α-estradiol, β-estradiol, estrone and ethinylestradiol) in transitional waters, i.e. Western Scheldt samples (brackish water). This research detected estrone and β-estradiol in the low ng L^{-1} range in water (not depicted on Figure 3), suggesting that steroidal EDCs could have already reached our marine environment.

However, it should be highlighted, that a larger series of emerging compounds should be identified, as the fate of many other steroidal EDCs, plasticizers and plastics additives is still poorly known, particularly following WWTP [116] and in the water in general [117]."

All EDCs will eventually degrade into other, less-well characterised compounds [118]. Biological degradation, generated by human, animal and microbial metabolism, will result in a wide range of transformation products as well [119]. Briefly, it can be concluded, that the marine environment is an important water body that may be suspected to contain numerous EDCs and their transformation and degradation products, and this at low ng.L^{-1} range. The evaluation of these EDCs in seawater, is a logic first step to assess how far these compounds have already reached our aquatic environment. This stresses the need of monitoring EDC residues in the marine waters. Therefore, this doctoral thesis focused on the analysis of EDCs in seawater, and not in sediments and biological species. Indeed, EDC residues in the environment have gained a growing public and scientific concern in recent years [120].

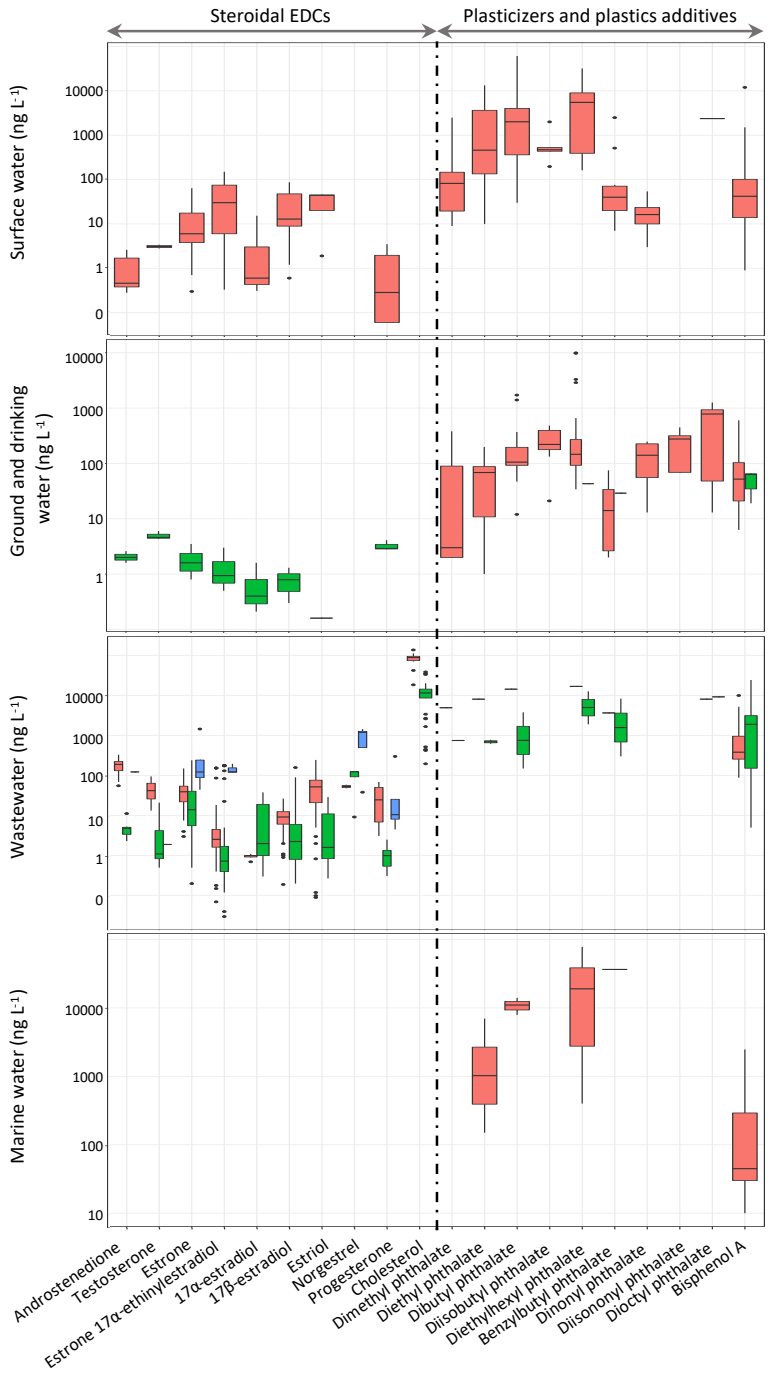


Figure 3. Concentrations of steroidal EDCs, plasticizers and plastics additives reported in the aquatic environment and presented as box-and-whisker plots and median (line box). The bottom and top of the box are the first and third quartiles of the data. Whiskers indicate minima and maxima, covering variability outside the upper and lower quartile. Green and red boxplots of panel 2 represent ground and drinking water, respectively. Red, green, and blue boxplots of panel 3 represent WWTP influent, WWTP effluent and animal farm wastewater, respectively. Only compounds for which more than 10 quantitative data points were found in literature, are included in this chart. Data sources: [89,90,109,110,112,113,126–131,91,132–141,92,142–151,95,152–161,96,162–167,97,99,100,107]**, from 2000-2015. Single horizontal lines represent 1 data point.**

2.3. Legal frameworks and directives

In response to public awareness and concerns about the health status of our aquatic environment, different legal frameworks and directives have been published [121–125], e.g. OSPAR (marine convention for the protection from Oslo to Paris), REACH, WFD (Water Framework Directive), Norman (directive) and CWA (U.S. Clean Water Act). An overview of the most important EDCs (in section 2.2) that are listed by these frameworks and directives can be consulted in

Table 1. Table 1 clearly demonstrates that each legal framework and or directive focusses on different EDCs, indicating that the current legal framework for the unambiguous identification of substances of emerging concern is unharmonized. Indeed, only 17β-estradiol and BPA have been included in most legislations and directives.

In light of this work, the European WFD and the Marine Strategy Framework Directive (MSFD, 2008/56/CE) were considered the most important. The WFD covers inland surface waters (e.g. rivers and lakes), transitional waters (e.g. coastal waters), and groundwater. For the marine environment, the MSFD has been established more recently and is closely tied to the EU WFD.

The WFD and MFSD overlap on coastal areas, since the WFD considers coastal waters as that are located one nautical mile out to sea. The WFD aims to achieve and ensure a “good water status” of all European water bodies and to prevent deterioration of the water status by 2027. The latest list of priority substances of the EU WFD comprises a total of 45 substances. For those substances, environmental quality standards (EQSs) are available. When exceedance of the EQSs is observed, member states are required to implement operational monitoring and to control the discharge and emission of priority substances to the aquatic body. Until now, only 17β-estradiol, 17β-ethinylestradiol and diethylhexyl phthalate have been included as steroidal EDCs in the European watch list for water quality monitoring.

Table 1. Overview of available legal frameworks and directives for EDCs in aquatic matrices.

EDC	OSPAR[1]	REACH[2]	WFD[3]	Norman[4]	CWA[5]
Steroidal EDCs					
17β-estradiol	A	A	A	A	
estrone	A	A		A	
17β-ethinylestradiol	A				
17-caproxyprogesterone	A				
17α-estradiol				A	
17α-ethinylestradiol	A		A	A	
cholesterol				A	
diethylstilbestrol	C			A	
estriol				A	
mestranol				A	
prednisolone				A	
dexamethasone				A	
nandrolone				A	
Plasticizers and plastics additives					
bisphenol A	B	A		A	
4-tert-octylphenol					
phenol					A
2,4,6-trichlorophenol					A
2,4-dichlorophenol					A
2,4-dimethylphenol					A
butyl benzyl phthalate	B	A		A	A
di-n-octyl phthalate	B			A	A
di-isodecyl phthalate				A	
diisononyl phthalate				A	
di-n-butyl phthalate		A		A	A
diethylhexyl phthalate		A	A		A
diethyl phthalate					A
dimethyl phthalate				A	A

[1] A = warrant further work by OSPAR, B=substances of concern according to OSPAR, but which are adequately addressed by EC initiatives or other international forums, C= threat to the marine environment
[2] A = recognised by REACH
[3] A = most priority substances
[4] A = emerging substances
[5] A = recognised as toxic and priority pollutant

3. CHEMICAL ANALYSIS OF EDCS IN THE AQUATIC ENVIRONMENT

In recent years, evolution in analytical instrumentation has resulted in a significant progress in the detection of micropollutants in the aquatic environment. Although considerable advancements have been achieved on the analytical side, the initial sampling and sample preparation steps still leave much to be desired. Within this literature overview, it is not the purpose to give a complete compilation of scientific research on the analysis of EDCs. Instead, our goal is to discuss the most frequently used extraction procedures and analytical instruments that enable the widest detection range of EDCs in the aquatic environment.

3.1. Sampling and sample preparation procedures

Sampling of environmental micropollutants encompasses analyte isolation and preconcentration. The combination of isolation and preconcentration, whether or not performed in one single step, results in two distinct types of aquatic sampling approaches, i.e. active and passive sampling. Active sampling – and in particularly the conventional grab sampling - requires mechanical work and/or energy in order to pass the contaminants in the water phase through an extraction device (appropriate sorbent phase). In the marine environment, the water column is commonly sampled using Niskin bottles [168], as depicted in Figure 4. Alternatively, passive sampling uses environmental advection and molecular diffusion to capture analytes in an extraction device directly exposed to the aquatic environment. Passive sampling shows a number of advantages compared to active sampling; i.e. less labour intensive (reduced cost) and more sensitive (lower detection limits) as there are no limitations with regard to the sample volume [169,170]. Additionally, passive samplers are effortless in transport and storage, because the analytes are already enriched on the sampler [171]. Nevertheless, there are some drawbacks related to the use of passive sampling. Most importantly, the effect of fluctuating environmental conditions on the analyte uptake needs to be taken into account [171].

Figure 4. Niskin bottle for sampling the water column at a certain depth.

Corrections for the latter can be made by using suitable performance reference compounds (PRCs) for the more non-polar compounds (log P> 4), which are comparable to the use of internal standards within active sampling. Moreover, within passive sampling, internal standards (or PRCs) are used for correcting the variation of the isolation and preconcentration of analytes, while in active samplers this can be used solely for correcting the variation of the preconcentration (and also the instrumental variation of the analysis). In addition, the selected deuterated standards for both strategies are chosen within the calibration range and covering the same polarity range as the envisaged target compounds.

Nevertheless, for the polar compounds (log P < 4) correcting for environmental fluctuations using PRCs is still challenging, and until now limitedly applied in passive sampling [172]. Furthermore, only a limited number of standard procedures for mostly non-polar compounds are available for passive samplers [173], while for grab sampling a plethora of directives and ISO guidelines exist (such as ISO 56667-1). The continuously increasing number of passive sampling designs and devices has also resulted in different handling procedures [174]. Despite of their individual advantages and disadvantages, using a combination of both sampling techniques will offer complementary information about the occurrence of contaminants in the water phase. Moreover, passive sampling provides information of compounds and levels covering the entire deployment duration (depending on the operation mode, further discussed in section 3.1.2.), whereas active samplers only provide information

about the total dissolved fraction at one specific point in time, unless of course a more elaborate active sampling campaign is designed. Consequently, dynamic concentrations (e.g. episodic inputs of contaminants and storms) are likely to be missed unless multiple samples are collected [175,176]. Additionally, passive samplers measure the freely dissolved or relevant concentration with regard to toxicological effects on biota (bioavailable concentration), whereas the measured fraction of active samplers depends on the sample pre-treatment step and allows quantification of both dissolved and particulate phases of contaminants in the water phase. Therefore, passive sampling opens up the opportunity of combining chemical analysis and toxicity testing, and thereby, forms the link between levels of contamination and their potential risk to the aquatic environment [177,178]. Currently, a plethora of extraction procedures are available for both active and passive sampling, as depicted in Figure 5. For the extraction of micropollutants from aquatic matrices, the use of polymeric materials and sorbents play a fundamental role within active and passive sampling. In active sampling, the use of polymeric sorbents is also known as solid-phase extraction (SPE). In passive sampling, polymeric devices are increasingly used to accumulate contaminants from environmental waters [179].

3.1.1. Active sampling

Following active sampling, a number of extraction techniques ranging from conventional liquid-liquid extraction (LLE), to more contemporary methods based on microextraction, may be used (Figure 5). Up to date, SPE is still the most frequently applied sample preparation and extraction technique [180,181]. SPE realizes an up-concentration of the analytes, but at the same time also produces cleaner extracts facilitating analysis [181]. Furthermore, improved retention of emerging compounds was obtained by the use of commercially available polymeric sorbents, mostly hydrophilic-hydrophobic balanced materials (e.g. Oasis HLB) and octadecyl silica bonded phases [182]. Commercially available polymers are generally suited for extracting a selective range of organic compounds, with each possessing a defined range of physico-chemical characteristics [183].

Nevertheless, the copolymer of divinylbenzene and N-vinyl pyrrolidone, better known as Oasis HLB^{TM}, is currently the most commonly used SPE-sorbent for extracting a physico-chemically diverse range of emerging micropollutants [184–188]. Prior to SPE, filtration of samples is usually performed to reduce the microbial degradation of analytes and to avoid clogging of SPE sorbents [189].

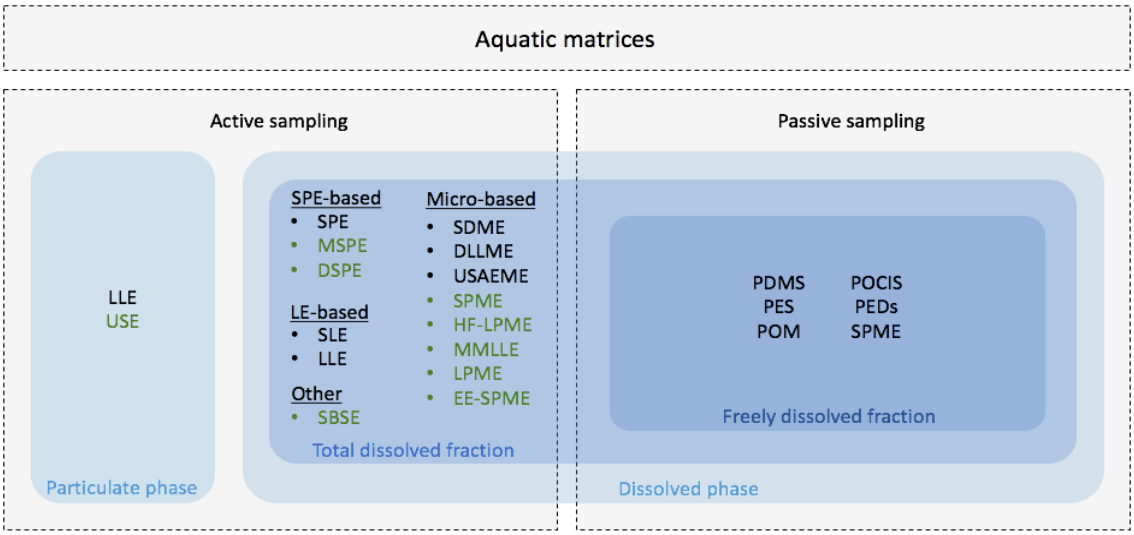


Figure 5. The available sample preparation techniques that have been reported in literature for measuring EDCs in aquatic matrices, classified by the sampling technique and the corresponding aquatic fractions. The sample preparation techniques highlighted in green are considered to be more environmentally friendly as less organic solvent is used during the extraction.

3.1.1.1. Steroidal EDCs

Overall, a number of studies have focused on the active sampling of steroidal EDCs in freshwater environments and particularly on the presence of oestrogens. Nevertheless, only a limited number of steroidal EDCs have been investigated so far, except for one study by Vulliet et al. (2011) in which 26 steroidal EDCs were measured simultaneously in surface and groundwaters [190]. Even more, the available standardized EPA methodology, for measuring steroidal EDCs in drinking water by SPE, includes only 12 steroidal EDCs [191]. Before extraction, aquatic samplers are mostly filtered, followed by a pH adjustment step [192–195]. The EDC extraction procedures reported so far for steroidal EDCs apply Oasis HLB^{TM} or Strata C18 SPE cartridges [190,194]. Upon sample loading, the sorbent materials are washed with

aqueous solutions, targeted steroidal EDCs are eluted with organic solvents, solvents are evaporated and the residue is reconstituted in a small volume of the appropriate injection solvent [196–198]. As many of the steroidal EDCs are present at trace levels in the aquatic environment (ng L^{-1}), large volumes of sampled water are usually needed to achieve reliable analytical performance [199]. Moreover, a (limited) number of sample precautions should be considered in order to avoid sample contamination. Some of the hormone compounds are biogenic and can be present on human skin or might be used as personal care products [189]. Furthermore, protective gloves must be worn at all times to minimize risk of contamination, while the collection of field blanks is essential to monitor for contamination during the sampling itself. Especially for quantitative analysis of cholesterol, it is important to avoid laboratory tissues or other paper-based products during sampling and extraction, because these products might contain cholesterol. More importantly, cholesterol is also present in human skin flakes and can even occur at substantial concentrations in both in and out-door dust [200].

3.1.1.2. Plasticizers and plastics additives

On top of those parameters of importance as described for the active sampling of steroidal EDCs, additional precautions should be taken for reducing systematic errors and false positives during active sampling of plasticizers and plastics additives [201,202]. Moreover, special attention must be paid to the pre-treatment and storage of samples that will be analysed on the presence of plasticizers and plastics additives, in particular phthalates, in aquatic matrices as well. It is self-evident that plastic materials should be avoided or screened for phthalates in order to minimize or avoid contamination when collecting and preparing samples. Even glassware should be cleaned using different solvents, e.g. ultra-pure acetone, dichloromethane, hexane and/or methanol, and heated over 400°C for at least 2 - 4 hours [203–207]. Following this, glass should be wrapped into calcinated aluminium foil to limit photochemical oxidation [203–207]. For active sampling, all sampling materials, including bottles, should be cleaned using 0.1 % HCl and Milli-Q water [208,209]. On the sampling site, storage containers (bottles) and materials should be rinsed with water from the sampling site

itself [210]. After sample collection by means of active sampling, water samples should be transferred to the rinsed bottles, and it is suggested to close them off using Teflon caps [110]. At present, most of the existing aquatic monitoring studies lack sufficiently specific knowledge regarding the suitability of water storage containers for phthalate sampling purposes. An overview of the used storage containers (since 2011), with reported phthalate contamination status, can be consulted in Table 2.

Migration of DEP and DEHP have been observed for almost all storage containers although, overall, a lot of discrepancies have been reported, with respect to e.g. polyethylene tetra phthalate (PET) bottles. More specifically, studies of Dévier et al. (2013) and Amiridou et al. (2011) have shown that the storage of samples in PET bottles at different temperatures and exposure times did not allow the detection of any of the phthalates of interest [210,213]. This is however contradicted by the results of a long-term study (11 months) published by Ustun et al. in 2015 [215], which observed that the main reason for phthalate contamination could be attributed to exposure time, rather than any other factor such as exposure to light, pH, storage temperature, humidity, and the use of additives. Nevertheless, several independent studies mention a significant increase in phthalates leaching from the storage container to the sample itself as a result of an increased temperature [214], whilst Keresztes (2013) observed leaching of phthalates at low sample pHs [212]. In contrast to the latter, several independent researchers recommended the acidification of samples for preservation [57].

Table 2. An overview of storage containers (since 2011) that were examined for possible phthalate contamination during sampling of aquatic matrices. Contamination with a target compound is depicted by a blue shaded area, its absence by a red shaded area. Underlined values represent the limits of detection (ng L^{-1}), the other values represent the limits of quantification (ng L^{-1}). Thereby, only the analytical limits of matrix-matched calibration curves were taken into consideration if possible, otherwise 'n.s.' i.e. not specified was mentioned.

Storage Device	Sample Type	Conditions	BBP	DBP	DAP	DbZP	DCHP	DEP	DEEP	DEHA	DEHP	DHP	DiBP	DiNP	DiPP	DnBP	DOP	DMP	DMEP	DPP	Reference
Fiberglass-reinforced plastic	Tap water	4°C 2d									1			1	1	1					[211]
Stainless steel	Tap water	4°C 2d									1			1	1	1					[211]
Glass - HDPE cap	Natural mineral	20°C 15-365d	190	230				330		80	460							10			[212]
Glass, metallic screw-cap	Natural mineral	20°C 15-365d	190	230				330		80	460							10			[212]
Glass metallic cap	Natural mineral	20°C 15-365d	190	230				330		80	460							10			[212]
PP - HDPE cap	Natural mineral	20°C 15-365d	190	230				330		80	460							10			[212]
PET	Natural mineral	40°C, 10d	30	20		30	20	30			10	20			20		10	30		20	[210]
PET	Natural mineral	15 – 40°C 15 – 30d	< 30	< 30				< 30			< 30						< 30	30			[213]
PET	Natural mineral	22°C 44-1200d	6.0	6.6				22.2			16		3.0					6.8			[214]
PET	Soft drink	30-334d	2000	4000				2000			7000			9000			2000	2000		3000	[215]
PET-HDEPE cap	Natural mineral	20°C 15-365d	190	230				330		80	460							10			[212]
PET	Unbuffered, treated by reversed osmosis	23-60°C 120d	250					130		130	130					130		130			[216]
PC	Natural mineral	15 – 40°C 15 – 30d	< 30	< 30				< 30			< 30						< 30	30			[213]
PC-HDPE cap	Natural mineral	20°C 15-365d	190	230				330		80	460							10			[212]
LDPE bag	Natural mineral	20°C 15-365d	190	230				330		80	460							10			[212]
HDPE	Natural mineral	20°C 15-365d	190	230				330		80	460							10			[212]
Commercial bottle (n.s.)	Natural mineral	7d	200	200	200			200	200		300							300	300		[217]

3.1.2. Passive sampling

Passive sampling techniques have been initially developed as an alternative for overcoming the challenges of active sampling, as extensively described under 3.1.1. Passive sampling relies on the diffusion of analytes from the aquatic phase to a receiving medium (i.e. passive sampling device), which is driven by the difference in chemical potential (described by Fick's first law) [218]. The uptake of analytes can be described by a partitioning process, which is assumed to follow first order kinetics. The partitioning process is initiated with a lag phase (not depicted in Figure 6), indicating the period of time before the start of the diffusion of the analytes towards the receiving medium, followed by a linear accumulation phase and a transition stage, to end with a curvilinear equilibrium stage with the surrounding aquatic environment (Figure 6). During the initial linear stage, contaminants sorb at a rate that is directly proportional to the aqueous concentration. As shown in Figure 6, passive samplers can be operated in the kinetic (linear) or equilibrium mode.

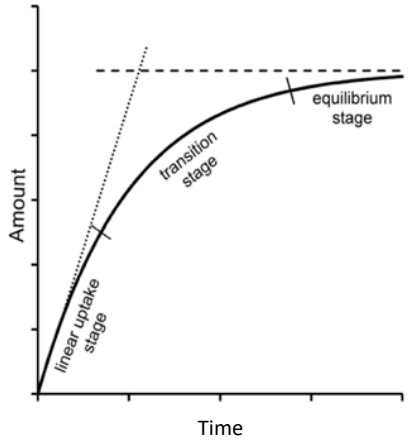


Figure 6. General accumulation profile of an analyte in a passive sampler as a function of time, thereby assuming a consistent (fixed) chemical concentration in the aquatic medium, showing the different uptake process stages (kinetic, transition and equilibrium), as reported by Smedes and Booij [173]**.**

Operation in the equilibrium mode, is typically used to provide a snap shot of the freely dissolved concentration, while kinetic samplers are designed to continuously accumulate analytes. Using kinetic samplers provides time-integrative organic compound levels (average concentrations), thus minimizing the risk of missing episodic inputs and variations [219]. The

uptake of analytes depends on the sampler design, the analyte's physico-chemical properties, and environmental variables (e.g. turbulence, temperature and fouling). Until now, a wide selection of passive sampling devices has been developed for sampling organic compounds. Passive samplers generally use a polymeric receiving phase, which is often combined with a limiting diffusion membrane. An overview of the composition of the most frequently applied passive samplers is depicted in Table 3. The choice of a specific passive sampler largely depends on the compound(s) or chemical class being targeted [220]. For the highly polar (Log P < 0) to medium non-polar (4 <log P < 6) compounds, Oasis HLB and Isolute ENV$^+$ have been frequently applied as receiving phase in different passive sampling designs [221].

The polar organic chemical integrative sampler (POCIS) and Chemcatcher sampler are mostly applied for sequestering polar (log P < 4) to semi-polar (log P ≈ 4) compounds [222–224]. SDB-RPS disks on their turn can retain ionizable polar (log P < 4) analytes through different mechanisms including π-π bonding, hydrogen bonding and Vander Waals and Coulomb interactions [225,226]. SDB-XC can be used for sampling more polar non-ionizable compounds [226], while C_{18}-disks are more prone to adsorb more hydrophobic compounds. For more hydrophobic compounds (log P > 4), semi-permeable membrane devices or single-phase samplers made of polymers have been proposed, including polydimethylsiloxane (PDMS), low-density polyethylene (LDPE) or polyoxymethylene (POM) [227–229]. The limiting diffusion membrane that is frequently put in front of the sorptive polymer, has three major roles: (1) improvement of sampler selectivity, (2) protection of the disk through prevention against biofouling and particle sticking, and (3) as additional mass transfer barrier to extend the integrative sampling period of linear uptake by decreasing the sampling rate. A variety of membranes have been applied for passive sampling purposes, especially for polar compounds. The most commonly used is polyether sulfone (PES).

Table 3. Comparison of different passive samplers that have been used for sampling organic micropollutants from aquatic matrices [227–230]**.**

	Membrane	Receiving phase
Chemcatcher	Polyethersulfone	SDB-RPS: Styrene divinylbenzene - reversed phase sulfonated
	Polysulfone	SDB-XC: Styrene divinylbenzene - exchange
	Polycarbonate	C_{18} Empore® disks
	LDPE	C_{18} Empore® disks
POCIS	Polyethersulfone	POCIS A: Triphasic sorbent mixture of Isolute ENV$^+$, polystyrene divinylbenzene and Ambersorb 1500 or 572 dispersed on S-X3 Biobeads
		POCIS B: Oasis HLB sorbent
PDMS	-	Polydimethylsiloxane
LDPE	-	Low-density polyethylene
POM	-	Polyoxymethylene

Its popularity can be attributed to its high hydrophilicity (log P of sampled components ranging between -2.6 to 5), which can be attributed to the presence of sulfonic acid groups [231,232]. Polysulfone membranes have also demonstrated good results for sampling of compounds with log P values ranging from 1 to 4 when coupled to SDB-RPS or SDB-XC disks. The use of polycarbonate (PC) and LDPE, together with C_{18}-disks, has shown its merits for sampling low polarity and neutral organic compounds [233,234]. Next to the surface chemistry of membranes, their pore size and thickness are also key parameters in the sampling process. Generally, the larger the membrane pore size, the easier organic compounds pass through, as indicated by a shorter lag-phase [225,232]. The retention of organic compounds by the membrane should be as weak as possible, to allow compounds to easily penetrate through the membrane [220].

3.1.2.1. Steroidal EDCs

Passive sampling of steroidal EDCs has been mainly investigated for oestrogens, while for androgens, progestogens and corticosteroids literature is scarce. SDB-RPS Empore disks have been shown suitable for the sequestration of 4 oestrogens and 1 androgen in WWTP

influent and effluent. The quantified EDC concentrations in passive samplers were, however, several times lower (factor 3 – 10) than those in grab samples. These lower concentrations can probably be attributed to the not sufficiently accurate calibration of the passive sampler uptake as a result of – but not necessarily limited to – biofouling, low flow rates and/or biodegradation [195]. Furthermore, in a different study, Vallejo et al. calibrated POCIS A for 6 oestrogens, 3 androgens and 1 progestagen. The concentrations determined by active sampling were lower (up to a factor of 10) (i.e. for estrone and testosterone) or comparable (i.e. for estradiol, ethinylestradiol, estriol and progesterone) than the concentrations obtained by POCIS [235]. A similar study by Zhang et al. investigated river water and WWTP effluent for 3 oestrogens (i.e. estrone, 17β-estradiol, 17α-ethynylestradiol) using the POCIS B configuration with PES and PS membranes [236]. The POCIS sampling rates were significantly higher (factor 5 to 10) when using PES instead of PS membranes. Furthermore, the sampling rates obtained in the field (R_s ranging between 0.3 and 0.9 L day^{-1}) were significantly higher (factor 5 to 10) than those obtained following laboratory calibration (R_s ranging between 0.02 and 0.2 L day^{-1}), which is in line with other studies [236,237]. Nevertheless, the use of sufficiently long exposure times (longer than 21 days) has been urged for monitoring steroidal EDCs in the field when using POCIS samplers [238]. Arditsoglou et al. observed that sampling rates for POCIS A and B (see Table 3) were comparable for the investigated oestrogens, ranging respectively from 0.11 to 0.21 L day^{-1} and 0.11 to 0.22 L day^{-1}. This study also concluded that POCIS provides reasonable estimates as compared to grab samples and thus a holistic picture of ambient concentrations of EDCs present in aquatic matrices. However, careful interpretation of the estimated concentrations is warranted, because environmental conditions (such as salt content, dissolved organic carbon content, flow rate, turbulence and pollution impact from different activities) can significantly differ as compared to the conditions applied during laboratory calibration studies [239].

3.1.2.2. Plasticizers and plastics additives

Even though passive sampling devices can provide more information on the entire deployment duration, only a limited number of passive sampling devices has been investigated for the measurement of phthalates in aquatic matrices. Until now, passive sampling techniques have only been used for the determination of di-phthalates. Such studies were performed *in situ* over a period of 28 consecutive days, although the exact applications differed in use and research goal.

The goal of a first study, reported by Posada-Ureta et al. (2016), was to calibrate three different passive samplers in terms of sampling rates, before deploying them in the aquatic environment. Calibration was performed on BBP and DOP, using polydimethylsiloxane stir bars (PDMS-stir bars) and PES and POM as receiving phase. They were investigated both with and without a membrane-enclosed sorptive coating (MESCO) by means of a dynamic continuous flow system, in which a continuous flow was fed to a controlled stirred tank with constant water volume. The deployed PDMS, PES and POM sheets without sorptive coating membrane enabled the successful determination of sampling rates. No sampling rates could be determined for POM when PES was used as receiving phase, while the use of a sorptive coating on the PDMS-stir bars resulted in a lack of fit during calibration [240].

The studies of Alvarez et al. (2014) and Maruya et al. (2014) discuss the direct deployment of passive samplers in bays, rivers and lagoons. More specific, the performance of POCIS, LDPE, and solid micro-extraction fibres was evaluated in binding DEP (log P = 2.7) and DEHP (log P = 7.6) [162,241]. The results demonstrated that only POCIS was able to reach a detection frequency exceeding 80% for DEP and DEHP, which is in line with the intended use of POCIS for efficient monitoring of both hydrophilic (log P < 3) and hydrophobic (log P > 3) compounds. The POCIS polarity duality can be explained by the presence of n-vinylpyrrolidone moieties on the divinylbenzene sorbent of Oasis HLB™. N-vinylpyrrolidone demonstrates a higher affinity for hydrophilic compounds, such as DEP, whereas divinylbenzene has a higher affinity for hydrophobic compounds like DEHP. However, the

latter three studies lack information on the analyte's sorption characteristics, including sampling rates (R_s) and sampler-water partitioning coefficients (K_{sw}).

Ultimately, it can be concluded that research on passive sampling devices so far mainly focussed on the monitoring of di-phthalates and their *in situ* behaviour. Passive sampling of primary and secondary phthalates in aquatic matrices has not been investigated yet. Finally, it should be noted that caution is warranted to prevent phthalate contamination during the deployment of passive samplers. The latter should be cleaned and conditioned with phthalate-free solvents. The ready to use passive samplers should be stored in Milli-Q water and this in an appropriate storage device (Table 2). After retrieval of the passive samplers, they should immediately be wrapped into calcinated aluminium foil.

3.2. Instrumental analysis

Highly sensitive and reliable analytical methods are needed for the analysis of micropollutants in the environment as they tend to prevail at nano levels [242]. Therefore, the use of chromatography hyphenated to mass spectrometry seems to be the platform of choice. Chromatography is a physical separation technique, whereby compounds selectively distribute between a mobile and stationary phase. Based on the physical state of the mobile phase, a differentiation between gas (GC) and liquid chromatography (LC) can be made. GC is a common type of chromatography applied in analytical chemistry for the separation of volatile compounds according to their volatility and polarity. LC enables the efficient separation of polar to non-polar compounds. As the before-mentioned EDCs are not sufficiently volatile for gas-chromatographic separation, the LC technique has been frequently used. Following chromatographic separation, the individual compounds can be detected by mass spectrometry. MS generates charged molecules or fragments thereof, after which the intensity of the latter ions is employed for sorting and detection, according to their mass-to-charge (*m/z*) ratio.

The majority of the instrumental methods developed so far, have been established using LC coupled to tandem mass spectrometry (LC-MS/MS), particularly using triple quadrupole (QqQ)

analyzers [243,244]. (Ultra)-high performance liquid chromatography ((U)-HPLC) coupled to tandem mass spectrometry (MS/MS) has shown its merit in meeting the demand for targeted residue analysis of organic compounds in aquatic matrices, and in confirming and quantifying down to the sub ng L^{-1} level [245]. In spite of the high selectivity and sensitivity reached, LC-MS/MS has some limitations regarding analysis of a large number of compounds or multi-class analysis. In MS/MS methods, the acquisition time of each transition limits the number of target analytes that can be analyzed. Despite of the latest evolution of QqQ instruments, encompassing low dwell times and allowing notable increases in the number of transitions within a run, analysis of thousands of contaminants that may potentially be present in waters is not possible using MS/MS detection.

High-resolution mass spectrometry (HRMS) instruments, such as time-of-flight (TOF) and Orbitrap, transcendent this major limitation of targeted MS/MS analysis. HRMS instruments provide high-quality throughput by combining sensitive full-scan data with high mass resolution, mass accuracy (measured to several decimal) and scanning speed [246]. As a result, the current trend in analytical chemistry is to screen and quantify as much as possible compounds in one analytical run. This provides considerable information about the occurrence of e.g. micropollutants (see section 2.2) and reduces the cost and analysis time [243,247–249]. Next to the mass spectrometric detection, the chromatographic separation is equally important. Indeed, an efficient chromatographic separation is essential to minimize or avoid matrix interferences, and to get reliable identification and reproducible results. The use of columns containing stationary-phases with particle sizes smaller than conventional high-performance liquid chromatography (HPLC), has emerged as an innovative powerful separation technique, i.e. ultra (U)-HPLC [250]. As a consequence, the system has to be designed to resist higher pressures during analysis. Improvement of the chromatographic separation towards UHPLC has led to shorter run times, improved sensitivity and better chromatographical separation of the analytes. For the investigation of multiple EDCs in the aquatic environment, the application of UHPLC-HRMS can play a crucial role [246].

3.2.1. Steroidal EDCs

Analysis of steroidal EDCs has been performed using LC-MS as well as GC-MS [136,251–255]. Prior to GC-MS analysis, derivatization of the hydroxyl or carbonyl moieties is carried out. This is done to enhance the volatility and thermal stability of the steroidal EDCs, and reduce the polarity by decreasing dipole-dipole interactions [256]. The most common derivatization technique for the steroidal EDCs consists of silylation using N-methyl-N-trimethylsilyl trifluoroacetamide [189]. Most steroidal EDCs can be analyzed directly, however, with LC-based analysis requiring less sample preparation. As a result thereof, LC-MS based methods for measuring steroidal EDCs have been increasingly applied, as their application does not require derivatization [136,253–255,257,258]. Separation has been regularly performed using reversed phased C_{18}-columns. In the context of separating steroidal EDCs, the use of biphenyl and phenyl-hexyl columns proved successful. The same was observed in clinical applications [198,259].

As can be deduced from Table 4, tandem mass spectrometry has been frequently applied upon LC separation. In environmental monitoring, electrospray ionization (ESI) has been mostly used as an ionisation source for steroidal EDCs, with as monitored ions in the positive and negative mode $[M+H]^+$ and $[M-H]^-$, respectively [260]. Other detectors, i.e. diode-array and fluorescence, have only rarely been used, due to their minor sensitivity and selectivity [100,256,261]. The use of HRMS instruments within environmental monitoring of steroidal EDCs has not been reported earlier. Although LC-MS/MS can provide a high analytical sensitivity and specificity, the number of compounds that can be measured is limited. Until now, for environmental analysis, a maximum of 26 steroidal EDCs could be determined in one analytical run.

3.2.2. Plasticizers and plastics additives

Until now, the analysis of plasticizers has mostly been performed by GC or LC coupled to MS, with a recent trend for LC separation [262]. GC often involves time consuming derivatization steps [263] and the analysis of high-molecular phthalates is limited as a result of their

intermediate volatility. LC is also more appropriate for analyzing metabolites (such as mono-phthalates) and degradation products of phthalates [57].

Therefore, LC-methods for measuring plasticizers have increasingly been used in recent years. An overview of the LC-MS methods that are currently available for plasticizers and plastics additives is depicted in Table 5. LC separation of plasticizers is generally carried out using C_{18}, C_{12} or C_8-silane columns relying on dispersive interaction forces [264]. Shorter chain lengths promote the strength of separating phthalate isomers [57]. Tandem mass spectrometry, using multiple reaction monitoring (MRM), selected ion monitoring (SIM) or full scan events is mainly used to detect separated phthalates (Table 5). The parent phthalates are generally detected in the positive ionization mode, while for the primary and secondary metabolites, the negative ionization is primarily used. In line with the sampling procedures, considerable attention must be paid to the possible occurrence of contamination originating from the analytical instrument itself, which is well reported by different technical notes, vendors and researchers [265,266]. Generally, contamination in LC systems originates from the used solvents and LC parts, such as tubes, injectors, valves, pumping systems and solvent degassers. Mass spectrometric contamination has also been observed for DBP, DOP, DEHP and DMP [267,268]. Additionally, Verge and Agnes et. al (2002) reported the contamination of vacuum o-rings employed in ESI-MS with phthalates [269].

Table 4. Hyphenated LC-based methods for the analysis of steroidal EDCs in aquatic matrices, i.e. marine water, surface water, wastewater and drinking water (n.s. = not specified).

LC type	Analytical column	Mobile phase	Additive	Ionization source	Detector system	MS scan type	Aquatic matrices	Number of steroidal EDCs	Ref.
HPLC	BetaBAsic C18 (150 x 2.1 mm, 3 µm)	Water/acetonitrile	N-methyl morpholine	ESI	MS/MS	SRM	Surface and waste water	4	[98]
HPLC	LiChrospher 100 RP18 (250 × 4 mm, 5 µm)	Water/acetonitrile	-	ESI	MS	SIM	Surface water	8	[135]
HPLC	Altima C18 (25 cm x 4.6 mm, 5 µm)	Water/acetonitrile	Ammonia	ESI	MS/MS	SRM	Surface and waste water	4	[97]
HPLC	Zorbax Eclipse XDB C18 (100 × 2.1 mm, 3.5 µm)	Water/acetonitrile	Formic acid	ESI	MS/MS	MRM	Surface and ground water	26	[88]
HPLC	Zorbax Eclipse XDB-C18 (150 × 4.6 mm, 5 µm)	Water/acetonitrile	Potassium phosphate	n.s.	DAD and FD	n.s.	Waste water	4	[100]
HPLC	Hypersil GOLD™ C18 (50 × 2.1 mm , 3 µm)	Water/methanol	Formic acid	APPI	MS/MS	SRM	Surface water	8	[96]
HPLC	Kromasil 100 C18 (25.0 × 0.46 cm, 5 µm)	Water/acetonitrile	Acetic acid	ESI	MS/MS	SRM	Waste water	11	[138]
HPLC	Zorbax Eclipse Plus column (3.0 × 100 mm, 3.5 µm)	Water/methanol	Formic acid and ammonium formate	ESI	MS/MS	MRM	Surface water	6	[133]
UHPLC	BEH C18 (140 x 1.0 mm, 1.7 µm)	Water/methanol	Ammonium acetate and acetic acid	ESI	MS/MS	MRM	Surface and waste water	3	[136]
UHPLC	BEH C18 column (100 × 2.1 mm, 1.7 µm)	Water/methanol	Formic acid	ESI	MS/MS	MRM	Surface and waste water	18	[127]
UHPLC	BEH C18 column (100 × 2.1 mm, 1.7 µm)	Water/methanol	Formic acid	ESI	MS/MS	MRM	Surface and waste water	23	[94]
UHPLC	Agilent Zorbax SB-C18 (100 × 3 mm, 1.8 µm)	Water/acetonitrile	Acetic and formic acid	ESI	MS/MS	MRM	Waste water	23	[93]

Table 5. LC-MS methods (since 2016) for the determination of phthalates in aquatic matrices, i.e. marine waters, surface waters, drinking water (including beverages) and wastewater (n.s. = not specified).

LC type	Analytical column	Mobile phase	Additive	Ionization source	MS system	MS scan type	Aquatic matrices	Number of phthalates	Ref.
HPLC	LiChrospher 100 RP-18 (4 x 250 mm, 5 µm)	Water/methanol		ESI	MS	Full scan	Surface Waters	5	[271]
HPLC	ZORBAX Eclipse XDB-C8 (2.1 x 50 mm, 3.5 µm)	Water/acetonitrile	Acetic Acid	ESI	MS	Full scan	Marine waters	3	[272]
HPLC	Agilent Eclipse XDB-C8 (4.6 × 150 mm, 5 µm)	Water/methanol		ESI	MS/MS	MRM	Drinking water	1	[273]
HPLC	Synergi RPMAX C12 (3 x 150 mm, 4 µm)	Water/methanol	Acetic Acid	ESI	MS/MS	MRM	Seawater	10	[274]
HPLC	Acclaim-C18 (4.5 x 250 mm, 5 µm)	Water/acetonitrile		ESI	MS/MS	MRM	Tea	6	[275]
HPLC	C18 Thermo fisher gold (2.1 x 100 mm, 5 µm)	Water/methanol	Formic Acid	ESI	MS/MS	n.s.	Bottled waters	8	[166]
HPLC	a Luna Phenyl-Hexyl (2 x 150 mm, 3 µm)	Water/methanol	Acetic Acid	ESI	MS/MS	n.s.	Wastewater	8	[276]
HPLC	LiChrospher 100 RP-18 (4 x 250 mm, 5-µm)	Water/methanol		ESI	MS/MS	SIM	River water	5	[154]
HPLC	Zorbax Eclipse XDB-C8 (2.1 x 50 mm, 3.5 µm)	Water/acetonitrile	Acetic Acid	ESI	MS/MS	SIM	Water	4	[277]
HPLC	LiChrospher 100 RP-18 (4 x 250 mm, 5 µm)	Water/ acetonitrile		APCI	MS	n.s.	Water	1	[278]
UHPLC	Betasil Phenyl (2.1 x 100 mm, 3µm)	Water/ acetonitrile	Acetic Acid	ESI	MS/MS	CID	Milk	6	[279]
UHPLC	Thermo Hypersil GOLD (2.1 x 50 mm, 1.9 µm)	Water/ acetonitrile	Phosphoric Acid	ESI	MS	Full scan	Water and wastewater	3	[280]
UHPLC	Acquity UPLC BEHC18 (2.1 x 50 mm, 1.7 µm)	Water/MeOH	Formic Acid	ESI	MS/MS	MRM	Distilled Beverages	7	[281]
UHPLC	Acquity UPLCs BEH Phenyl (2.1 x 50 mm, 1.7 µm)	Water/ acetonitrile	Acetic Acid	ESI	MS/MS	MRM	Only instrumental method	11	[282]

4. CONCEPTUAL FRAMEWORK AND OBJECTIVES OF THIS STUDY

"Nowadays, growing public and scientific concerns exist regarding the widespread occurrence of potential EDCs in the aquatic environment. In this context, the most important potential EDCs are the steroid hormones (interact at very low concentrations with the hormonal system) and plasticizers (extensively used in polymeric products and coatings), which could interfere with endocrine systems of living organisms. As shown in section 2.2., until now limited knowledge is available on the presence of steroidal EDCs, plasticizers and plastics additives in seawater. Nevertheless, the threat of these contaminants to aquatic organisms and thus the aquatic ecosystem as a whole can be enormous, as a result of the fact that they are continuously exposed [24]. The EDCs prevailing in seawater can potentially be accumulated by aquatic organisms, i.e. ranging from lower (e.g. earthworms) to higher organisms (e.g. fish). The consumption of these higher organisms can result in human exposure to EDCs [270]. Despite their known and possibly unknown toxicological effects, limited efforts have been made so far to investigate the prevalence of steroidal EDCs, plasticizers and plastics additives on a quantitative basis in seawater. As such, the first step, prior to assessing aquatic organisms through the food chain, would be to investigate whether or not aquatic organisms are exposed to these EDCs in the marine environment. To study the fate, effects, and environmental and human risks posed by potential EDCs in aquatic ecosystems, information regarding their presence in the marine environment is indeed urgently needed. Therefore, **the main goal of the present study was to investigate the prevalence of steroidal EDCs, plasticizers and plastics additives in the Belgian marine environment, i.e. seawater**. During this doctoral research, the main focus was to investigate potential EDCs, of which their prevalence was not or only limited investigated in the BPNS. Therefore, perfluorinated chemicals and organophosphate flame retardants were excluded from this work, as these potential EDCs were already investigated in the BPNS [70,71]. Moreover, the study of Wille et al. showed that the majority of perfluorinated compounds were not detected [71], while flame retardants were extensively detected in the BPNS [70]. The study of the prevalence of

steroidal EDCs, plasticizers and plastics additives implies the need for new and reliable analytical methods targeting a broad range of EDCs in seawaters and resulted as a consequence in the doctoral framework that is depicted in Figure 7. The framework consists out of 3 main research chapters, which is preceded by a general introduction and followed by a broader discussion on future perspectives. In the introduction (Chapter I), an elaborate literature review is presented on the fate of EDCs in water and on the current status of the available analytical methodologies for their (active and passive) sampling and instrumental analysis. Until present, reports on the analysis of a broad range of EDCs using active and passive samplers are scarce. Therefore, Chapter II describes the **development and validation of new HRMS-based analytical methods** for the quantification of a wide range of steroidal EDCs, plasticizers and plastics additives in aqueous matrices, i.e. fresh and salt water. This second chapter formed the basis for a third chapter, in which the use of a 'novel' and unexamined sorbent, i.e. **hydrophilic DVB, is investigated for passive sampling purposes** in seawater. The sorption behavior of a large number of emerging compounds is investigated in depth. Moreover, chapter III determined the sorbent-water partitioning coefficient (K_{sw}) and the effect of increasing concentration and environmental parameters on the K_{sw}. Chapter IV comprises the application of all newly developed methodologies from chapters II and III in the marine environment by performing a field study. Thereby, the status of **EDC contamination of the Belgian marine environment** is described for 2016 and 2017. Even more, the potential negative effects of their prevalence and ecotoxicological implications are briefly framed. Finally, the overall outcome and results of this doctoral thesis is discussed. General conclusions are drawn and future research recommendations are formulated (Chapter V).

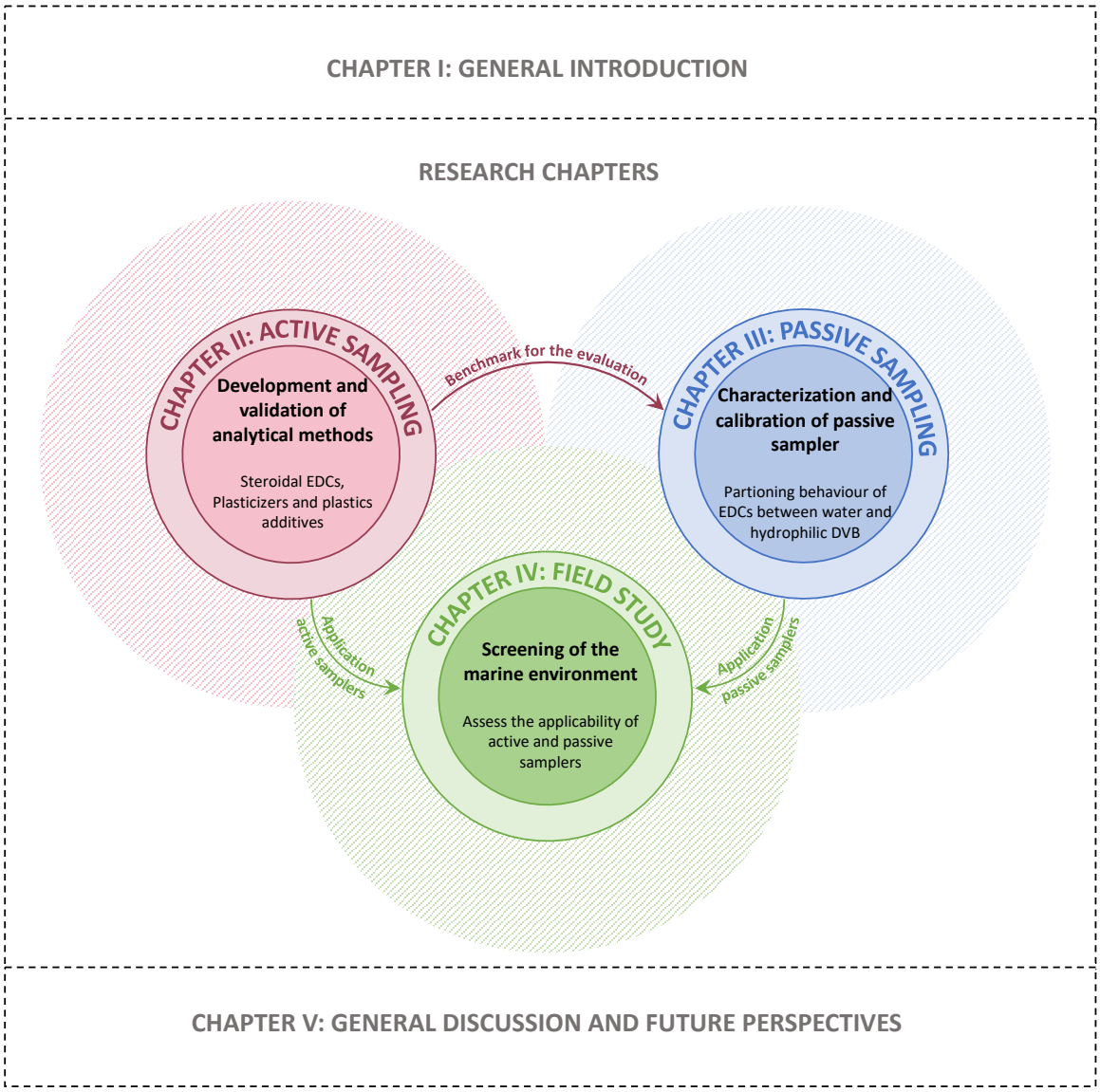


Figure 7. Conceptual framework of this doctoral thesis encompassing general introduction (I), the three major research chapters, i.e. (II) active sampling, (III) passive sampling, (IV) field study on EDC contamination of the BPNS, and the general discussion and future perspectives (V).

REFERENCES

[1] M.O. Barbosa, N.F.F. Moreira, A.R. Ribeiro, M.F.R. Pereira, A.M.T. Silva, Occurrence and removal of organic micropollutants: An overview of the watch list of EU Decision 2015/495, Water Res. 94 (2016) 257–279. doi:10.1016/j.watres.2016.02.047.

[2] N.J. Rowan, Defining established and emergingmicrobial risks in the aquatic environment: Current knowledge, implications, and outlooks, Int. J. Microbiol. (2011). doi:10.1155/2011/462832.

[3] R.P. Schwarzenbach, B.I. Escher, K. Fenner, T.B. Hofstetter, C.A. Johnson, U. Von Gunten, et al., The challenge of micropollutants in aquatic systems, Science (80-.). 313 (2006) 1072–1077. doi:10.1126/science.1127291.

[4] Q. Bu, D. Wang, Z. Wang, Review of screening systems for prioritizing chemical substances, Crit. Rev. Environ. Sci. Technol. 43 (2013) 1011–1041. doi:10.1080/10934529.2011.627030.

[5] T.T. Schug, A.F. Johnson, L.S. Birnbaum, T. Colborn, L.J. Guillette, D.P. Crews, et al., Minireview: Endocrine Disruptors: Past Lessons and Future Directions, Mol. Endocrinol. 30 (2016) 833–847. doi:10.1210/me.2016-1096.

[6] R.J. Aulerich, R.K. Ringer, Current status of PCB toxicity to mink, and effect on their reproduction., Arch. Environ. Contam. Toxicol. 6 (1977) 279–92. http://www.ncbi.nlm.nih.gov/pubmed/409355 (accessed May 4, 2019).

[7] J.A. McLachlan, Environmental signaling: What embryos and evolution teach us about endocrine disrupting chemicals, Endocr. Rev. 22 (2001) 319–341. doi:10.1210/edrv.22.3.0432.

[8] J. A. McLachlan, Estrogens in the Environment, (1980). doi:10.2307/3433868.

[9] J.A. Mclachlan, R.R. Newbold, Estrogens and Development II, 1987. https://pdfs.semanticscholar.org/0bbd/288d44088572e10048572085edfca4f166d0.pdf (accessed May 5, 2019).

[10] J.J. Bull, W.H.N. Gutzke, D. Crews, Sex reversal by estradiol in three reptilian orders, Gen. Comp. Endocrinol. 70 (1988) 425–428. doi:10.1016/0016-6480(88)90117-7.

[11] D. Crews, T. Wibbels, W.H.N. Gutzke, Action of sex steroid hormones on temperature-induced sex determination in the snapping turtle (Chelydra serpentina), Gen. Comp. Endocrinol. 76 (1989) 159–166. doi:10.1016/0016-6480(89)90042-7.

[12] A.M. Soto, M. V. Maffini, C.M. Schaeberle, C. Sonnenschein, Strengths and weaknesses of in vitro assays for estrogenic and androgenic activity, Best Pract. Res. Clin. Endocrinol. Metab. 20 (2006) 15–33. doi:10.1016/j.beem.2005.09.001.

[13] S. AM, J. H, W. JW, S. C, p-Nonyl-phenol: an estrogenic xenobiotic released from “modified” polystyrene, Environ. Health Perspect. 92 (1991) 167–173. doi:10.1289/ehp.9192167.

[14] S. Jobling, T. Reynolds, R. White, M.G. Parker, P. John, J.P. Sumpter, A Variety of Environmentally Persistent Chemicals , Including Some Phthalate Plasticizers , Are Weakly Estrogenic, Environ. Heal. (2010). https://ehp.niehs.nih.gov/doi/pdf/10.1289/ehp.95103582 (accessed May 5, 2019).

[15] M. Schlumpf, B. Cotton, M. Conscience, V. Haller, B. Steinmann, W. Lichtensteiger, In vitro and in vivo estrogenicity of UV screens, Environ. Health Perspect. 109 (2001) 239–244. doi:10.1289/ehp.01109239.

[16] F.S. vom Saal, B.G. Timms, M.M. Montano, P. Palanza, K.A. Thayer, S.C. Nagel, et al., Prostate enlargement in mice due to fetal exposure to low doses of estradiol or diethylstilbestrol and opposite effects at high doses., Proc. Natl. Acad. Sci. U. S. A. 94 (1997) 2056–61. www.pnas.org. (accessed May 5, 2019).

[17] C. Colborn, T, Clement, Chemically-Induced Alterations in Sexual and Functional Development: The Wildlife/Human Connection, Adv. Mod. Environemental Toxicol. 21 (1992) 403. http://agris.fao.org/agris-search/search.do?recordID=US9545328 (accessed May 5, 2019).

[18] J.A. McLachlan, K.S. Korach, Symposium on estrogens in the environment, III, in: Environ. Health Perspect., 1995: pp. 3–4. doi:10.1289/ehp.95103s73.

[19] International Programme on Chemical Safety, Global Assessment of the State-of-the-Science of Endocrine Disruptors, WHO/PCS/EDC/02.2. (2002). https://inemg.com/download-437319.pdf (accessed May 5, 2019).

[20] E. Diamanti-Kandarakis, J.-P. Bourguignon, L.C. Giudice, R. Hauser, G.S. Prins, A.M. Soto, et al., Endocrine-disrupting chemicals: an Endocrine Society scientific statement., Endocr. Rev. 30 (2009) 293–342. doi:10.1210/er.2009-0002.

[21] \r A Bergman, J.J. Heindel, S. Jobling, K.A. Kidd, R.T. Zoeller, State of the science of endocrine disrupting chemicals 2012: an assessment of the state of the science of endocrine disruptors prepared by a group of experts …, (2013) 260.

[22] N. Scholz, Briefing Commission proposals on identifying endocrine disruptors, 2016. http://www.europarl.europa.eu/RegData/etudes/BRIE/2016/586629/EPRS_BRI(2016)586629_EN.pdf (accessed May 5, 2019).

[23] US-EPA, Special report on environmental endocrine disruption: an effects assessment and analyses, Rep. N° EPA/630/R-96/012. (1997) 120. doi:EPA/630/R-96/012.

[24] A. Garcia-Rodriguez, V. Matamoros, C. Fontas, V. Salvado, The ability of biologically based wastewater treatment systems to remove emerging organic contaminants--a review, Environ. Sci. Pollut. Res. Int. 21 (2014) 11708–11728. doi:10.1007/s11356-013-2448-5.

[25] J.P. Nash, D.E. Kime, L.T.M. Van der Ven, P.W. Wester, F. Brion, G. Maack, et al., Long-term exposure to environmental concentrations of the pharmaceutical ethynylestradiol causes reproductive failure in fish, Environ. Health Perspect. 112 (2004) 1725–1733. doi:10.1289/ehp.7209.

[26] J.P. Laurenson, R. a Bloom, S. Page, N. Sadrieh, Ethinyl estradiol and other human pharmaceutical estrogens in the aquatic environment: a review of recent risk assessment data., AAPS J. 16 (2014) 299–310. doi:10.1208/s12248-014-9561-3.

[27] V. Christen, S. Hickmann, B. Rechenberg, K. Fent, Highly active human pharmaceuticals in aquatic systems: A concept for their identification based on their mode of action, Aquat. Toxicol. 96 (2010) 167–181. doi:10.1016/j.aquatox.2009.11.021.

[28] C.R. Tyler, S. Jobling, Roach, Sex, and Gender-Bending Chemicals: The Feminization of Wild Fish in English Rivers, Bioscience. 58 (2008) 1051. doi:10.1641/B581108.

[29] K. Fent, Progestins as endocrine disrupters in aquatic ecosystems: Concentrations, effects and risk assessment, Environ. Int. 84 (2015) 115–130. doi:10.1016/j.envint.2015.06.012.

[30] D.J. Caldwell, F. Mastrocco, T.H. Hutchinson, R. Länge, D. Heijerick, C. Janssen, et al., Derivation of an aquatic predicted no-effect concentration for the synthetic hormone, 17α-ethinyl estradiol, Environ. Sci. Technol. 42 (2008) 7046–7054. doi:10.1021/es800633q.

[31] J. Fick, R.H. Lindberg, J. Parkkonen, B. Arvidsson, M. Tysklind, D.G. Joakim Larsson, Therapeutic levels of levonorgestrel detected in blood plasma of fish: results from screening rainbow trout exposed to treated sewage effluents, Environ. Sci. Technol. 44 (2010) 2661–2666. doi:10.1021/es903440m.

[32] J. Zeilinger, T. Steger-Hartmann, E. Maser, S. Goller, R. Vonk, R. L??nge, Effects of synthetic gestagens on fish reproduction, Environ. Toxicol. Chem. 28 (2009) 2663–2670. doi:10.1897/08-485.1.

[33] S. Liu, H. Chen, X.-R. Xu, S.-S. Liu, K.-F. Sun, J.-L. Zhao, et al., Steroids in marine aquaculture farms surrounding Hailing Island, South China: Occurrence, bioconcentration, and human dietary exposure, Sci. Total Environ. 502 (2015) 400–407. doi:10.1016/j.scitotenv.2014.09.039.

[34] M.R. Servos, D.T. Bennie, B.K. Burnison, A. Jurkovic, R. McInnis, T. Neheli, et al., Distribution of estrogens, 17β-estradiol and estrone, in Canadian municipal wastewater treatment plants, Sci. Total Environ. 336 (2005) 155–170. doi:10.1016/J.SCITOTENV.2004.05.025.

[35] J. Joseph, M. Parr, Synthetic Androgens as Designer Supplements, Curr. Neuropharmacol. 13 (2015) 89–100. doi:10.2174/1570159X13666141210224756.

[36] J. Li, F. Al-Azzawi, Mechanism of androgen receptor action, Maturitas. 63 (2009) 142–148. doi:10.1016/j.maturitas.2009.03.008.

[37] J. You, H. Zhao, Z. Sun, Y. Suo, G. Chen, 10-Ethyl-acridine-2-sulfonyl Chloride: A New Derivatization Agent for Enhancement of Atmospheric Pressure Chemical Ionization of Estrogens in Urine, Chromatographia. 70 (2009) 45–55. doi:10.1365/s10337-009-1101-4.

[38] H. Tapiero, K. D., G., Polyphenols: do they play a role in the prevention of human pathologies? Biomedicine & Pharmacotherapy, 56 (2002) 200–207. https://ac.els-cdn.com/S0753332202001786/1-s2.0-S0753332202001786-main.pdf?_tid=ab20040d-223e-4f71-a0fb-55d1928daf4a&acdnat=1546423697_961d37e16e9f7ed72230ff5137895af3 (accessed January 2, 2019).

[39] P.A.M. Onteleone, R.A.I. Oime, A.N.R.G. Enazzani, M.A.M. Aj, Plasma Levels of Neuroactive Steroids Are Increased in Untreated Women With Anorexia Nervosa or Bulimia Nervosa, Journals.Lww.Com. 68 (2001) 62–68. https://journals.lww.com/psychosomaticmedicine/Abstract/2001/01000/Plasma_Levels_of_Neuroactive_Steroids_Are.8.aspx (accessed November 19, 2018).

[40] Z. Hochberg, R. Chayen, N. Reiss, Z. Falik, A. Makler, M. Munichor, et al., Clinical, biochemical, and genetic findings in a large pedigree of male and female patients with 5α-reductase 2 deficiency, J. Clin. Endocrinol. Metab. 81 (1996) 2821–2827. doi:10.1210/jc.81.8.2821.

[41] C. Madeddu, G. Gramignano, C. Floris, G. Murenu, G. Sollai, A. Macciò, Role of inflammation and oxidative stress in post-menopausal oestrogen-dependent breast cancer, J. Cell. Mol. Med. 18 (2014) 2519–2529. doi:10.1111/jcmm.12413.

[42] K. Yoon, S.J. Kwack, H.S. Kim, B.M. Lee, Estrogenic endocrine-disrupting chemicals: Molecular mechanisms of actions on putative human diseases, J. Toxicol. Environ. Heal. - Part B Crit. Rev. 17 (2014) 127–174. doi:10.1080/10937404.2014.882194.

[43] P. Labadie, H. Budzinski, Determination of steroidal hormone profiles along the Jalle d'Eysines River (near Bordeaux, France), Environ. Sci. Technol. 39 (2005) 5113–5120. doi:10.1021/es048443g.

[44] R. Länge, T.H. Hutchinson, C.P. Croudace, F. Siegmund, H. Schweinfurth, P. Hampe, et al., Effects of the synthetic estrogen 17α-ethinylestradiol on the life-cycle of the fathead minnow (Pimephales promelas), Environ. Toxicol. Chem. 20 (2001) 1216–1227. doi:10.1002/etc.5620200610.

[45] U. Fuhrmann, R. Krattenmacher, E.P. Slater, K.-H. Fritzemeier, The novel progestin drospirenone and its natural counterpart progesterone: Biochemical profile and antiandrogenic potential, Contraception. 54 (1996) 243–251. doi:10.1016/S0010-7824(96)00195-3.

[46] R. Sitruk-Ware, New progestogens: A review of their effects in perimenopausal and postmenopausal women, Drugs and Aging. 21 (2004) 865–883. doi:10.2165/00002512-200421130-00004.

[47] A.C. Gore, V.A. Chappell, S.E. Fenton, J.A. Flaws, A. Nadal, G.S. Prins, et al., Executive Summary to EDC-2: The Endocrine Society's second Scientific Statement on endocrine-disrupting chemicals, Endocr. Rev. 36 (2015) 593–602. doi:10.1210/er.2015-1093.

[48] H. Bártíková, R. Podlipná, L. Skálová, Veterinary drugs in the environment and their toxicity to plants, Chemosphere. 144 (2016) 2290–2301. doi:10.1016/j.chemosphere.2015.10.137.

[49] S.K. Wasser, S. Papageorge, C. Foley, J.L. Brown, Excretory fate of estradiol and progesterone in the African elephant (Loxodonta africana) and patterns of fecal steroid concentrations throughout the estrous cycle, Gen. Comp. Endocrinol. 102 (1996) 255–262. doi:10.1006/gcen.1996.0067.

[50] M. Heistermann, M. Agil, A. Büthe, J.K. Hodges, Metabolism and excretion of oestradiol-17β and progesterone in the Sumatran rhinoceros (Dicerorhinus sumatrensis), Anim. Reprod. Sci. 53 (1998) 157–172. doi:10.1016/S0378-4320(98)00111-0.

[51] E.K. Shanle, W. Xu, Endocrine disrupting chemicals targeting estrogen receptor signaling: Identification and mechanisms of action, Chem. Res. Toxicol. 24 (2011) 6–19. doi:10.1021/tx100231n.

[52] W.-L. Ma, B. Subedi, K. Kannan, The Occurrence of Bisphenol A, Phthalates, Parabens and Other Environmental Phenolic Compounds in House Dust: A Review, Curr. Org. Chem. 18 (2014) 2182–2199. doi:10.2174/1385272819666140804230205.

[53] E. Fasano, F. Bono-Blay, T. Cirillo, P. Montuori, S. Lacorte, Migration of phthalates, alkylphenols, bisphenol A and di(2-ethylhexyl)adipate from food packaging, Food Control. 27 (2012) 132–138. doi:10.1016/j.foodcont.2012.03.005.

[54] D. Amiridou, D. Voutsa, Alkylphenols and phthalates in bottled waters, J. Hazard. Mater. 185 (2011) 281–286. doi:10.1016/j.jhazmat.2010.09.031.

[55] J. Sampson, D. de Korte, DEHP-plasticised PVC: relevance to blood services*, Transfus. Med. 21 (2011) 73–83. doi:10.1111/j.1365-3148.2010.01056.x.

[56] H. Frederiksen, N.E. Skakkebæk, A.M. Andersson, Metabolism of phthalates in humans, Mol. Nutr. Food Res. 51 (2007) 899–911. doi:10.1002/mnfr.200600243.

[57] S. Net, A. Delmont, R. Sempéré, A. Paluselli, B. Ouddane, Reliable quantification of phthalates in environmental matrices (air, water, sludge, sediment and soil): A review, Sci. Total Environ. 515–516 (2015) 162–180. doi:10.1016/j.scitotenv.2015.02.013.

[58] M. Wittassek, H.M. Koch, J. Angerer, T. Brüning, Assessing exposure to phthalates - The human biomonitoring approach, Mol. Nutr. Food Res. 55 (2011) 7–31. doi:10.1002/mnfr.201000121.

[59] R. Hauser, A.M. Calafat, Phthalates and human health, Occup. Environ. Med. 62 (2005) 806–818. doi:10.1136/oem.2004.017590.

[60] H.M. Koch, A.M. Calafat, Human body burdens of chemicals used in plastic manufacture, Philos. Trans. R. Soc. B Biol. Sci. 364 (2009) 2063–2078. doi:10.1098/rstb.2008.0208.

[61] M.J. Silva, E. Samandar, J.L. Preau, J.A. Reidy, L.L. Needham, A.M. Calafat, Quantification of 22 phthalate metabolites in human urine, J. Chromatogr. B Anal. Technol. Biomed. Life Sci. 860 (2007) 106–112. doi:10.1016/j.jchromb.2007.10.023.

[62] T.T. Bui, G. Giovanoulis, A.P. Cousins, J. Magnér, I.T. Cousins, C.A. de Wit, Human exposure, hazard and risk of alternative plasticizers to phthalate esters, Sci. Total Environ. 541 (2016) 451–467. doi:10.1016/j.scitotenv.2015.09.036.

[63] B.C. Blount, M.J. Silva, S.P. Caudill, L.L. Needham, J.L. Pirkle, E.J. Sampson, et al., Levels of seven urinary phthalate metabolites in a human reference population., Environ. Health Perspect. 108 (2000) 979–82. doi:10.1289/ehp.00108979.

[64] H.M. Koch, B. Rossbach, H. Drexler, J. Angerer, Internal exposure of the general population to DEHP and other phthalates - Determination of secondary and primary phthalate monoester metabolites in urine, Environ. Res. 93 (2003) 177–185. doi:10.1016/S0013-9351(03)00083-5.

[65] M.J. Silva, D.B. Barr, J.A. Reidy, N.A. Malek, C.C. Hodge, S.P. Caudill, et al., Urinary levels of seven phthalate metabolites in the U.S. population from the National Health and Nutrition Examination Survey (NHANES) 1999-2000, Environ. Health Perspect. 112 (2004) 331–338. doi:10.1289/ehp.6723.

[66] A.M. Calafat, L.L. Needham, Factors affecting the evaluation of biomonitoring data for human exposure assessment, in: Int. J. Androl., John Wiley & Sons, Ltd, 2008: pp. 139–143. doi:10.1111/j.1365-2605.2007.00826.x.

[67] A. Ramesh Kumar, P. Sivaperumal, Analytical methods for the determination of biomarkers of exposure to phthalates in human urine samples, TrAC - Trends Anal. Chem. 75 (2016) 151–161. doi:10.1016/j.trac.2015.06.008.

[68] X. Hu, Y. Gu, W. Huang, D. Yin, Phthalate monoesters as markers of phthalate contamination in wild marine organisms, Environ. Pollut. 218 (2016) 410–418. doi:10.1016/j.envpol.2016.07.020.

[69] C.A. Richter, L.S. Birnbaum, F. Farabollini, R.R. Newbold, B.S. Rubin, C.E. Talsness, et al., In vivo effects of bisphenol A in laboratory rodent studies, Reprod. Toxicol. 24 (2007) 199–224. doi:10.1016/j.reprotox.2007.06.004.

[70] C.A. de Wit, D. Herzke, K. Vorkamp, Brominated flame retardants in the Arctic environment - trends and new candidates, Sci. Total Environ. 408 (2010) 2885–2918.

doi:10.1016/j.scitotenv.2009.08.037.
[71] X.L. Cao, C. Perez-Locas, G. Dufresne, G. Clement, S. Popovic, F. Beraldin, et al., Concentrations of bisphenol a in the composite food samples from the 2008 Canadian total diet study in Quebec City and dietary intake estimates, Food Addit. Contam. - Part A Chem. Anal. Control. Expo. Risk Assess. 28 (2011) 791–798. doi:10.1080/19440049.2010.513015.
[72] A. Goodson, W. Summerfield, I. Cooper, Survey of bisphenol A and bisphenol F in canned foods, Food Addit. Contam. 19 (2002) 796–802. doi:10.1080/02652030210146837.
[73] T. Geens, T.Z. Apelbaum, L. Goeyens, H. Neels, A. Covaci, Intake of bisphenol A from canned beverages and foods on the Belgian market, Food Addit. Contam. - Part A Chem. Anal. Control. Expo. Risk Assess. 27 (2010) 1627–1637. doi:10.1080/19440049.2010.508183.
[74] G.O. Noonan, L.K. Ackerman, T.H. Begley, Concentration of bisphenol a in highly consumed canned foods on the U.S. market, J. Agric. Food Chem. 59 (2011) 7178–7185. doi:10.1021/jf201076f.
[75] D. Aschberger, K., Castello, P., Hoekstra, E., Karakitsios, S., Munn, S., Pakalin, S. and Sarigiannis, Bisphenol A and baby bottles: challenges and perspectives, 2010. doi:10.1093/jxb/ern171.
[76] G. Schönfelder, W. Wittfoht, H. Hopp, C.E. Talsness, M. Paul, I. Chahoud, Parent Bisphenol A Accumulation in the Human Maternal–Fetal–Placental Unit, 2002. http://ehpnet1.niehs.nih.gov/docs/2002/110pA703-A707schonfelder/abstract.html (accessed January 28, 2019).
[77] A. Ballesteros-Gómez, S. Rubio, D. Pérez-Bendito, Analytical methods for the determination of bisphenol A in food, J. Chromatogr. A. 1216 (2009) 449–469. doi:10.1016/J.CHROMA.2008.06.037.
[78] T. Geens, D. Aerts, C. Berthot, J.-P. Bourguignon, L. Goeyens, P. Lecomte, et al., A review of dietary and non-dietary exposure to bisphenol-A, Food Chem. Toxicol. 50 (2012) 3725–3740. doi:10.1016/J.FCT.2012.07.059.
[79] J.B. Matthews, K. Twomey, T.R. Zacharewski, In vitro and in vivo interactions of bisphenol A and its metabolite, bisphenol A glucuronide, with estrogen receptors ?? and ??, Chem. Res. Toxicol. 14 (2001) 149–157. doi:10.1021/tx0001833.
[80] R.W. Snyder, S.C. Maness, K.W. Gaido, F. Welsch, S.C.J. Sumner, T.R. Fennell, Metabolism and disposition of bisphenol A in female rats, Toxicol. Appl. Pharmacol. 168 (2000) 225–234. doi:10.1006/taap.2000.9051.
[81] R. Viñas, R.M. Goldblum, C.S. Watson, Rapid estrogenic signaling activities of the modified (chlorinated, sulfonated, and glucuronidated) endocrine disruptor bisphenol A, Endocr. Disruptors. 1 (2013) e25411. doi:10.4161/endo.25411.
[82] P.J. Meffin, D.M. Zilm, J.R. Veenendaal, K. Eckhardt, K. Bosslet, H.K. Kroemer, Reduced clofibric acid clearance in renal dysfunction is due to a futile cycle., J. Pharmacol. Exp. Ther. 227 (1983) 732–8. http://www.ncbi.nlm.nih.gov/pubmed/6655566 (accessed November 18, 2018).
[83] G. Ginsberg, D.C. Rice, Does rapid metabolism ensure negligible risk from bisphenol A?, Environ. Health Perspect. 117 (2009) 1639–1643. doi:10.1289/ehp.0901010.
[84] H.H. Le, E.M. Carlson, J.P. Chua, S.M. Belcher, Bisphenol A is released from polycarbonate drinking bottles and mimics the neurotoxic actions of estrogen in developing cerebellar neurons, Toxicol. Lett. 176 (2008) 149–156. doi:10.1016/j.toxlet.2007.11.001.
[85] L.N. Vandenberg, R. Hauser, M. Marcus, N. Olea, W. V. Welshons, Human exposure to bisphenol A (BPA), Reprod. Toxicol. 24 (2007) 139–177. doi:10.1016/J.REPROTOX.2007.07.010.
[86] W. Dekant, W. Völkel, Human exposure to bisphenol A by biomonitoring: Methods, results and assessment of environmental exposures, Toxicol. Appl. Pharmacol. 228 (2008) 114–134. doi:10.1016/j.taap.2007.12.008.
[87] C.J. Vörösmarty, P.B. McIntyre, M.O. Gessner, D. Dudgeon, A. Prusevich, P. Green, et al., Global threats to human water security and river biodiversity, Nature. 467 (2010) 555–561. doi:10.1038/nature09440.
[88] E. Vulliet, L. Wiest, R. Baudot, M.-F. Grenier-Loustalot, Multi-residue analysis of steroids at sub-

ng/L levels in surface and ground-waters using liquid chromatography coupled to tandem mass spectrometry, J. Chromatogr. A. 1210 (2008) 84–91. doi:10.1016/j.chroma.2008.09.034.

[89] P. Hohenblum, O. Gans, W. Moche, S. Scharf, G. Lorbeer, Monitoring of selected estrogenic hormones and industrial chemicals in groundwaters and surface waters in Austria, Sci. Total Environ. 333 (2004) 185–193. doi:10.1016/j.scitotenv.2004.05.009.

[90] T.E. Félix-Cañedo, J.C. Durán-Álvarez, B. Jiménez-Cisneros, The occurrence and distribution of a group of organic micropollutants in Mexico City's water sources, Sci. Total Environ. 454–455 (2013) 109–118. doi:10.1016/j.scitotenv.2013.02.088.

[91] A. Colin, C. Bach, C. Rosin, J.-F. Munoz, X. Dauchy, Is Drinking Water a Major Route of Human Exposure to Alkylphenol and Bisphenol Contaminants in France?, Arch. Environ. Contam. Toxicol. 66 (2014) 86–99. doi:10.1007/s00244-013-9942-0.

[92] S.D. Kim, J. Cho, I.S. Kim, B.J. Vanderford, S.A. Snyder, Occurrence and removal of pharmaceuticals and endocrine disruptors in South Korean surface, drinking, and waste waters, Water Res. 41 (2007) 1013–1021. doi:10.1016/J.WATRES.2006.06.034.

[93] S. Liu, G.-G. Ying, J.-L. Zhao, F. Chen, B. Yang, L.-J. Zhou, et al., Trace analysis of 28 steroids in surface water, wastewater and sludge samples by rapid resolution liquid chromatography–electrospray ionization tandem mass spectrometry, J. Chromatogr. A. 1218 (2011) 1367–1378. doi:10.1016/j.chroma.2011.01.014.

[94] H. Chang, Y. Wan, S. Wu, Z. Fan, J. Hu, Occurrence of androgens and progestogens in wastewater treatment plants and receiving river waters: Comparison to estrogens, Water Res. 45 (2011) 732–740. doi:10.1016/j.watres.2010.08.046.

[95] M.P. Fernandez, M.G. Ikonomou, I. Buchanan, An assessment of estrogenic organic contaminants in Canadian wastewaters, Sci. Total Environ. 373 (2007) 250–269. doi:10.1016/j.scitotenv.2006.11.018.

[96] L. Viglino, K. Aboulfadl, M. Prévost, S. Sauvé, Analysis of natural and synthetic estrogenic endocrine disruptors in environmental waters using online preconcentration coupled with LC-APPI-MS/MS, Talanta. 76 (2008) 1088–1096. doi:10.1016/j.talanta.2008.05.008.

[97] C. Baronti, R. Curini, G. D'Ascenzo, A. Di Corcia, A. Gentili, R. Samperi, Monitoring natural and synthetic estrogens at activated sludge sewage treatment plants and in a receiving river water, Environ. Sci. Technol. 34 (2000) 5059–5066. doi:10.1021/es001359q.

[98] C.-Y. Chen, T.-Y. Wen, G.-S. Wang, H.-W. Cheng, Y.-H. Lin, G.-W. Lien, Determining estrogenic steroids in Taipei waters and removal in drinking water treatment using high-flow solid-phase extraction and liquid chromatography/tandem mass spectrometry, Sci. Total Environ. 378 (2007) 352–365. doi:10.1016/J.SCITOTENV.2007.02.038.

[99] B. Pauwels, H. Noppe, H. De Brabander, W. Verstraete, Comparison of Steroid Hormone Concentrations in Domestic and Hospital Wastewater Treatment Plants, J. Environ. Eng. 134 (2008) 933–936. doi:10.1061/(ASCE)0733-9372(2008)134:11(933).

[100] J. Martín, D. Camacho-Muñoz, J.L. Santos, I. Aparicio, E. Alonso, Occurrence of pharmaceutical compounds in wastewater and sludge from wastewater treatment plants: Removal and ecotoxicological impact of wastewater discharges and sludge disposal, J. Hazard. Mater. 239–240 (2012) 40–47. doi:10.1016/J.JHAZMAT.2012.04.068.

[101] A.C. Belfroid, A. Van Der Horst, A.D. Vethaak, A.J. Schäfer, G.B.J. Rijs, J. Wegener, et al., Analysis and occurrence of estrogenic hormones and their glucuronides in surface water and waste water in The Netherlands, Sci. Total Environ. 225 (1999) 101–108. doi:10.1016/S0048-9697(98)00336-2.

[102] G. D'Ascenzo, A. Di Corcia, A. Gentili, R. Mancini, R. Mastropasqua, M. Nazzari, et al., Fate of natural estrogen conjugates in municipal sewage transport and treatment facilities, Sci. Total Environ. 302 (2003) 199–209. doi:10.1016/S0048-9697(02)00342-X.

[103] C. Desbrow, E.J. Routledge, G.C. Brighty, J.P. Sumpter, M. Waldock, Identification of estrogenic chemicals in STW effluent. 1. Chemical fractionation and in vitro biological screening, Environ. Sci. Technol. 32 (1998) 1549–1558. doi:10.1021/es9707973.

[104] E.J. Routledge, D. Sheahan, C. Desbrow, G.C. Brighty, M. Waldock, J.P. Sumpter, Identification of estrogenic chemicals in STW effluent. 2. In vivo responses in trout and roach, Environ. Sci. Technol. 32 (1998) 1559–1565. doi:10.1021/es970796a.

[105] T.. Ternes, M. Stumpf, J. Mueller, K. Haberer, R.-D. Wilken, M. Servos, Behavior and occurrence of estrogens in municipal sewage treatment plants — I. Investigations in Germany, Canada and Brazil, Sci. Total Environ. 225 (1999) 81–90. doi:10.1016/S0048-9697(98)00334-9.

[106] L.S. Shore, M. Shemesh, Estrogen as an Environmental Pollutant, Bull. Environ. Contam. Toxicol. 97 (2016) 447–448. doi:10.1007/s00128-016-1873-9.

[107] D. Gao, Z. Li, Z. Wen, N. Ren, Occurrence and fate of phthalate esters in full-scale domestic wastewater treatment plants and their impact on receiving waters along the Songhua River in China, Chemosphere. 95 (2014) 24–32. doi:10.1016/j.chemosphere.2013.08.009.

[108] B. Petrie, L. Lopardo, K. Proctor, J. Youdan, R. Barden, B. Kasprzyk-Hordern, Assessment of bisphenol-A in the urban water cycle, Sci. Total Environ. 650 (2019) 900–907. doi:10.1016/j.scitotenv.2018.09.011.

[109] R. Loos, G. Hanke, G. Umlauf, S.J. Eisenreich, LC-MS-MS analysis and occurrence of octyl- and nonylphenol, their ethoxylates and their carboxylates in Belgian and Italian textile industry, waste water treatment plant effluents and surface waters, Chemosphere. 66 (2007) 690–699. doi:10.1016/j.chemosphere.2006.07.060.

[110] C. Dargnat, M.-J. Teil, M. Chevreuil, M. Blanchard, Phthalate removal throughout wastewater treatment plant: Case study of Marne Aval station (France), Sci. Total Environ. 407 (2009) 1235–1244. doi:10.1016/J.SCITOTENV.2008.10.027.

[111] X. Wu, H. Hong, X. Liu, W. Guan, L. Meng, Y. Ye, et al., Graphene-dispersive solid-phase extraction of phthalate acid esters from environmental water, Sci. Total Environ. 444 (2013) 224–230. doi:10.1016/j.scitotenv.2012.11.060.

[112] S. Liu, G.-G. Ying, R.-Q. Zhang, L.-J. Zhou, H.-J. Lai, Z.-F. Chen, Fate and occurrence of steroids in swine and dairy cattle farms with different farming scales and wastes disposal systems, Environ. Pollut. 170 (2012) 190–201. doi:10.1016/J.ENVPOL.2012.07.016.

[113] S. Liu, G.-G. Ying, L.-J. Zhou, R.-Q. Zhang, Z.-F. Chen, H.-J. Lai, Steroids in a typical swine farm and their release into the environment, Water Res. 46 (2012) 3754–3768. doi:10.1016/J.WATRES.2012.04.006.

[114] M. Adeel, X. Song, Y. Wang, D. Francis, Y. Yang, Environmental impact of estrogens on human, animal and plant life: A critical review, Environ. Int. 99 (2017) 107–119. doi:10.1016/j.envint.2016.12.010.

[115] H. Noppe, T. Verslycke, E. De Wulf, K. Verheyden, E. Monteyne, P. Van Caeter, et al., Occurrence of estrogens in the Scheldt estuary: A 2-year survey, Ecotoxicol. Environ. Saf. 66 (2007) 1–8. doi:10.1016/j.ecoenv.2006.04.005.

[116] Q. Wu, J.C.W. Lam, K.Y. Kwok, M.M.P. Tsui, P.K.S. Lam, Occurrence and fate of endogenous steroid hormones, alkylphenol ethoxylates, bisphenol A and phthalates in municipal sewage treatment systems, J. Environ. Sci. 61 (2017) 49–58. doi:10.1016/j.jes.2017.02.021.

[117] Q. Zhang, J.L. Zhao, G.G. Ying, Y.S. Liu, C.G. Pan, Emission estimation and multimedia fate modeling of seven steroids at the river basin scale in China, Environ. Sci. Technol. 48 (2014) 7982–7992. doi:10.1021/es501226h.

[118] L. Ferrando-Climent, N. Collado, G. Buttiglieri, M. Gros, I. Rodriguez-Roda, S. Rodriguez-Mozaz, et al., Comprehensive study of ibuprofen and its metabolites in activated sludge batch experiments and aquatic environment, Sci. Total Environ. 438 (2012) 404–413. doi:10.1016/j.scitotenv.2012.08.073.

[119] E.R. Christensen, A. Li, Physical and chemical processes in the aquatic environment, n.d. https://books.google.be/books?hl=nl&lr=&id=tY9ZBAAAQBAJ&oi=fnd&pg=PA268&dq=physical+and+chemical+processes+in+the+aquatic+environment&ots=ufOR7jpOPN&sig=B-RsRoLuDx-_LpoAm4-wU69bDas#v=onepage&q=physical and chemical processes in the aquatic environment&f=false (accessed November 8, 2018).

[120] E.J. Tiedeken, A. Tahar, B. McHugh, N.J. Rowan, Monitoring, sources, receptors, and control measures for three European Union watch list substances of emerging concern in receiving waters – A 20 year systematic review, Sci. Total Environ. 574 (2017) 1140–1163. doi:10.1016/j.scitotenv.2016.09.084.

[121] Ospar, OSPAR Commission, Mar. Ecol. Prog. Ser. (2014). https://www.ospar.org/about/publications (accessed December 4, 2018).

[122] I.J. Allan, B. Vrana, R. Greenwood, G.A. Mills, B. Roig, C. Gonzalez, A "toolbox" for biological and chemical monitoring requirements for the European Union's Water Framework Directive, in: Talanta, Elsevier, 2006: pp. 302–322. doi:10.1016/j.talanta.2005.09.043.

[123] N.A. Slobodnik, NORMAN Position Paper Collection, exchange and interpretation of data on emerging substances Towards a harmonised approach for collection and interpretation of data on emerging substances in support of European environmental policies, 2014. www.norman-network.net (accessed December 4, 2018).

[124] European Chemicals Agency, Understanding Reach, ECHA - Eur. Chem. Agency. (2015) 2015. https://echa.europa.eu/regulations/reach/understanding-reach%5Cnhttp://echa.europa.eu/web/guest/regulations/reach/understanding-reach.

[125] C. Copeland, Clean water act: A summary of the law, in: Atmos. Depos. Pollut. EPA, 2012. doi:10.4172/2332-0915.1000133.

[126] S. Liu, G.G. Ying, J.L. Zhao, F. Chen, B. Yang, L.J. Zhou, et al., Trace analysis of 28 steroids in surface water, wastewater and sludge samples by rapid resolution liquid chromatography-electrospray ionization tandem mass spectrometry, J. Chromatogr. A. 1218 (2011) 1367–1378. doi:10.1016/j.chroma.2011.01.014.

[127] H. Chang, S. Wu, J. Hu, M. Asami, S. Kunikane, Trace analysis of androgens and progestogens in environmental waters by ultra-performance liquid chromatography-electrospray tandem mass spectrometry, J. Chromatogr. A. 1195 (2008) 44–51. doi:10.1016/j.chroma.2008.04.055.

[128] Z. Fan, S. Wu, H. Chang, J. Hu, Behaviors of Glucocorticoids, Androgens and Progestogens in a Municipal Sewage Treatment Plant: Comparison to Estrogens, Environ. Sci. Technol. 45 (2011) 2725–2733. doi:10.1021/es103429c.

[129] E. Vulliet, L. Wiest, R. Baudot, M.F. Grenier-Loustalot, Multi-residue analysis of steroids at sub-ng/L levels in surface and ground-waters using liquid chromatography coupled to tandem mass spectrometry, J. Chromatogr. A. 1210 (2008) 84–91. doi:10.1016/j.chroma.2008.09.034.

[130] H.M. Kuch, K. Ballschmiter, Determination of endocrine-disrupting phenolic compounds and estrogens in surface and drinking water by HRGC-(NCI)-MS in the picogram per liter range, Environ. Sci. Technol. 35 (2001) 3201–3206. doi:10.1021/es010034m.

[131] S. Aguayo, M.J. Muñoz, A. de la Torre, J. Roset, E. de la Peña, M. Carballo, Identification of organic compounds and ecotoxicological assessment of sewage treatment plants (STP) effluents, Sci. Total Environ. 328 (2004) 69–81. doi:10.1016/J.SCITOTENV.2004.02.013.

[132] S. Zorita, L. Mårtensson, L. Mathiasson, Occurrence and removal of pharmaceuticals in a municipal sewage treatment system in the south of Sweden, Sci. Total Environ. 407 (2009) 2760–2770. doi:10.1016/j.scitotenv.2008.12.030.

[133] N.H. Torres, M.M. Aguiar, L.F.R. Ferreira, J.H.P. Américo, Â.M. Machado, E.B. Cavalcanti, et al., Detection of hormones in surface and drinking water in Brazil by LC-ESI-MS/MS and ecotoxicological assessment with Daphnia magna, Environ. Monit. Assess. 187 (2015) 379. doi:10.1007/s10661-015-4626-z.

[134] S. Rocha, V.F. Domingues, C. Pinho, V.C. Fernandes, C. Delerue-Matos, P. Gameiro, et al., Occurrence of bisphenol A, estrone, 17β-estradiol and 17α-ethinylestradiol in Portuguese rivers, Bull. Environ. Contam. Toxicol. 90 (2013) 73–78. doi:10.1007/s00128-012-0887-1.

[135] S. Rodriguez-Mozaz, M.J. López De Alda, D. Barceló, Monitoring of estrogens, pesticides and bisphenol A in natural waters and drinking water treatment plants by solid-phase extraction-liquid chromatography-mass spectrometry, in: J. Chromatogr. A, Elsevier, 2004: pp. 85–92. doi:10.1016/j.chroma.2004.06.040.

[136] B. Petrie, J. Youdan, R. Barden, B. Kasprzyk-Hordern, Multi-residue analysis of 90 emerging contaminants in liquid and solid environmental matrices by ultra-high-performance liquid chromatography tandem mass spectrometry, J. Chromatogr. A. 1431 (2016) 64–78. doi:10.1016/j.chroma.2015.12.036.

[137] S.X.L. Goh, A. Duarah, L. Zhang, S.A. Snyder, H.K. Lee, Online solid phase extraction with liquid chromatography–tandem mass spectrometry for determination of estrogens and glucocorticoids in water, J. Chromatogr. A. 1465 (2016) 9–19. doi:10.1016/J.CHROMA.2016.08.040.

[138] M. Pedrouzo, F. Borrull, E. Pocurull, R.M. Marcé, Estrogens and their conjugates: Determination in water samples by solid-phase extraction and liquid chromatography–tandem mass spectrometry, Talanta. 78 (2009) 1327–1331. doi:10.1016/J.TALANTA.2009.02.005.

[139] M. Pedrouzo, F. Borrull, E. Pocurull, R.M. Marcé, Presence of pharmaceuticals and hormones in waters from sewage treatment plants, Water. Air. Soil Pollut. 217 (2011) 267–281. doi:10.1007/s11270-010-0585-8.

[140] A. Mousa, C. Basheer, A. Rahman Al-Arfaj, Application of electro-enhanced solid-phase microextraction for determination of phthalate esters and bisphenol A in blood and seawater samples, Talanta. 115 (2013) 308–313. doi:10.1016/j.talanta.2013.05.011.

[141] G.C.C. Yang, C.H. Yen, C.L. Wang, Monitoring and removal of residual phthalate esters and pharmaceuticals in the drinking water of Kaohsiung City, Taiwan, J. Hazard. Mater. 277 (2014) 53–61. doi:10.1016/j.jhazmat.2014.03.005.

[142] G. Prokůpková, K. Holadová, J. Poustka, J. Hajšlová, Development of a solid-phase microextraction method for the determination of phthalic acid esters in water, Anal. Chim. Acta. 457 (2002) 211–223. doi:10.1016/S0003-2670(02)00020-X.

[143] H.S. Shin, C.H. Park, S.J. Park, H. Pyo, Sensitive determination of bisphenol A in environmental water by gas chromatography with nitrogen-phosphorus detection after cyanomethylation, J. Chromatogr. A. 912 (2001) 119–125. doi:10.1016/S0021-9673(01)00570-2.

[144] X. Li, G.G. Ying, H.C. Su, X.B. Yang, L. Wang, Simultaneous determination and assessment of 4-nonylphenol, bisphenol A and triclosan in tap water, bottled water and baby bottles, Environ. Int. 36 (2010) 557–562. doi:10.1016/j.envint.2010.04.009.

[145] A. Belfroid, M. Van Velzen, B. Van der Horst, D. Vethaak, Occurrence of bisphenol A in surface water and uptake in fish: Evaluation of field measurements, Chemosphere. 49 (2002) 97–103. doi:10.1016/S0045-6535(02)00157-1.

[146] C. Basheer, H.K. Lee, K.S. Tan, Endocrine disrupting alkylphenols and bisphenol-A in coastal waters and supermarket seafood from Singapore, Mar. Pollut. Bull. 48 (2004) 1161–1167. doi:10.1016/S0213-9111(00)71916-9.

[147] X. Zheng, B.-T. Zhang, Y. Teng, Distribution of phthalate acid esters in lakes of Beijing and its relationship with anthropogenic activities., Sci. Total Environ. 476–477 (2014) 107–13. doi:10.1016/j.scitotenv.2013.12.111.

[148] W. He, N. Qin, X. Kong, W. Liu, Q. He, H. Ouyang, et al., Spatio-temporal distributions and the ecological and health risks of phthalate esters (PAEs) in the surface water of a large, shallow Chinese lake, Sci. Total Environ. 461–462 (2013) 672–680. doi:10.1016/j.scitotenv.2013.05.049.

[149] M.J. Teil, M. Blanchard, C. Dargnat, K. Larcher-Tiphagne, M. Chevreuil, Occurence of phthalate diesters in rivers of the Paris district (France), Hydrol. Process. 21 (2007) 2515–2525. doi:10.1002/hyp.6484.

[150] D.W. Kolpin, E.T. Furlong, M.T. Meyer, E.M. Thurman, S.D. Zaugg, L.B. Barber, et al., Pharmaceuticals, hormones, and other organic wastewater contaminants in U.S. streams, 1999-2000: A national reconnaissance, Environ. Sci. Technol. 36 (2002) 1202–1211. doi:10.1021/es011055j.

[151] J.H. Kang, F. Kondo, Bisphenol A in the Surface Water and Freshwater Snail Collected from Rivers Around a Secure Landfill, Bull. Environ. Contam. Toxicol. 76 (2006) 113–118.

doi:10.1007/s00128-005-0896-4.

[152] *,† Toshinari Suzuki, ‡ Yoshio Nakagawa, ‡ Ichiro Takano, ‡ and Kumiko Yaguchi, K. Yasuda†, Environmental Fate of Bisphenol A and Its Biological Metabolites in River Water and Their Xeno-estrogenic Activity, (2004). doi:10.1021/ES030576Z.

[153] G.R. Boyd, J.M. Palmeri, S. Zhang, D.A. Grimm, Pharmaceuticals and personal care products (PPCPs) and endocrine disrupting chemicals (EDCs) in stormwater canals and Bayou St. John in New Orleans, Louisiana, USA, Sci. Total Environ. 333 (2004) 137–148. doi:10.1016/j.scitotenv.2004.03.018.

[154] R. Céspedes, S. Lacorte, A. Ginebreda, D. Barceló, Chemical monitoring and occurrence of alkylphenols, alkylphenol ethoxylates, alcohol ethoxylates, phthalates and benzothiazoles in sewage treatment plants and receiving waters along the ter River basin (Catalonia, N. E. Spain), in: Anal. Bioanal. Chem., Springer-Verlag, 2006: pp. 992–1000. doi:10.1007/s00216-006-0448-8.

[155] S. Kleywegt, V. Pileggi, P. Yang, C. Hao, X. Zhao, C. Rocks, et al., Pharmaceuticals, hormones and bisphenol A in untreated source and finished drinking water in Ontario, Canada - Occurrence and treatment efficiency, Sci. Total Environ. 409 (2011) 1481–1488. doi:10.1016/j.scitotenv.2011.01.010.

[156] V.A. Santhi, N. Sakai, E.D. Ahmad, A.M. Mustafa, Occurrence of bisphenol A in surface water, drinking water and plasma from Malaysia with exposure assessment from consumption of drinking water, Sci. Total Environ. 427–428 (2012) 332–338. doi:10.1016/j.scitotenv.2012.04.041.

[157] M.T. Das, P. Ghosh, I.S. Thakur, Intake estimates of phthalate esters for South Delhi population based on exposure media assessment, Environ. Pollut. 189 (2014) 118–125. doi:10.1016/J.ENVPOL.2014.02.021.

[158] Y.B. Luo, Q.W. Yu, B.F. Yuan, Y.Q. Feng, Fast microextraction of phthalate acid esters from beverage, environmental water and perfume samples by magnetic multi-walled carbon nanotubes, Talanta. 90 (2012) 123–131. doi:10.1016/j.talanta.2012.01.015.

[159] X. Wu, H. Hong, X. Liu, W. Guan, L. Meng, Y. Ye, et al., Graphene-dispersive solid-phase extraction of phthalate acid esters from environmental water, Sci. Total Environ. 444 (2013) 224–230. doi:10.1016/j.scitotenv.2012.11.060.

[160] C.C. Lee, L.Y. Jiang, Y.L. Kuo, C.Y. Hsieh, C.S. Chen, C.J. Tien, The potential role of water quality parameters on occurrence of nonylphenol and bisphenol A and identification of their discharge sources in the river ecosystems, Chemosphere. 91 (2013) 904–911. doi:10.1016/j.chemosphere.2013.02.006.

[161] X.L. Cao, J. Corriveau, Determination of bisphenol a in water by isotope dilution headspace solid-phase microextraction and gas chromatography/mass spectrometry without derivatization, J. AOAC Int. 91 (2008) 622–629. doi:10.1016/j.chroma.2007.11.095.

[162] D.A. Alvarez, K.A. Maruya, N.G. Dodder, W. Lao, E.T. Furlong, K.L. Smalling, Occurrence of contaminants of emerging concern along the California coast (2009 – 10) using passive sampling devices, Mar. Pollut. Bull. 81 (2014) 347–354. doi:10.1016/j.marpolbul.2013.04.022.

[163] F. Zeng, K. Cui, Z. Xie, M. Liu, Y. Li, Y. Lin, et al., Occurrence of phthalate esters in water and sediment of urban lakes in a subtropical city, Guangzhou, South China, Environ. Int. 34 (2008) 372–380. doi:10.1016/j.envint.2007.09.002.

[164] Y. Liu, Z. Chen, J. Shen, Occurrence and removal characteristics of phthalate esters from typical water sources in northeast China, J. Anal. Methods Chem. 2013 (2013) 419349. doi:10.1155/2013/419349.

[165] S. Net, D. Dumoulin, R. El-Osmani, S. Rabodonirina, B. Ouddane, Case study of PAHs, Me-PAHs, PCBs, phthalates and pesticides contamination in the Somme River water, France, Int. J. Environ. Res. 8 (2014) 1159–1170. https://ijer.ut.ac.ir/article_809_24fc56c34d8b0f38ffc5b152c55c28bb.pdf (accessed November 8, 2018).

[166] A.J. Al Khatib, M. Muhammad, A. Adamu, ANALYSIS OF PHTHALATE PLASTICIZER IN JORDANIAN BOTTLED WATERS BY LIQUID CHROMATOGRAPHY-TANDEM MASS SPECTROPHOTOMETRY (LC-MS / MS), Eur. Sci. Journal, ESJ. 10 (2014) 271–282. https://eujournal.org/index.php/esj/article/view/3596 (accessed March 7, 2018).

[167] D.A. Alvarez, P.E. Stackelberg, J.D. Petty, J.N. Huckins, E.T. Furlong, S.D. Zaugg, et al., Comparison of a novel passive sampler to standard water-column sampling for organic contaminants associated with wastewater effluents entering a New Jersey stream, Chemosphere. 61 (2005) 610–622. doi:10.1016/j.chemosphere.2005.03.023.

[168] Z. Dai, H. Zhang, Q. Zhou, Y. Tian, T. Chen, C. Tu, et al., Occurrence of microplastics in the water column and sediment in an inland sea affected by intensive anthropogenic activities, Environ. Pollut. 242 (2018) 1557–1565. doi:10.1016/J.ENVPOL.2018.07.131.

[169] B. Zabiegała, A. Kot-Wasik, M. Urbanowicz, J. Namieśnik, Passive sampling as a tool for obtaining reliable analytical information in environmental quality monitoring, Anal. Bioanal. Chem. 396 (2010) 273–296. doi:10.1007/s00216-009-3244-4.

[170] B. Vrana, F. Smedes, R. Prokeš, R. Loos, N. Mazzella, C. Miege, et al., An interlaboratory study on passive sampling of emerging water pollutants, TrAC - Trends Anal. Chem. 76 (2016) 153–165. doi:10.1016/j.trac.2015.10.013.

[171] J. Namieśnik, B. Zabiegała, A. Kot-Wasik, M. Partyka, A. Wasik, Passive sampling and/or extraction techniques in environmental analysis: A review, Anal. Bioanal. Chem. 381 (2005) 279–301. doi:10.1007/s00216-004-2830-8.

[172] C. Harman, I.J. Allan, P.S. Bäuerlein, The challenge of exposure correction for polar passive samplers the PRC and the POCIS, Environ. Sci. Technol. 45 (2011) 9120–9121. doi:10.1021/es2033789.

[173] F. Smedes, K. Booij, Guidelines for passive sampling of hydrophobic contaminants in water using silicone rubber samplers International Council for the Exploration of the Sea Conseil International pour l'Exploration de la Mer, Ices Tech. Mar. E Environ. Sci. 52 (2012). www.ices.dk (accessed December 17, 2018).

[174] N. Morin, C. Miège, M. Coquery, J. Randon, Chemical calibration, performance, validation and applications of the polar organic chemical integrative sampler (POCIS) in aquatic environments, TrAC Trends Anal. Chem. 36 (2012) 144–175. doi:10.1016/J.TRAC.2012.01.007.

[175] I.K. Wittmer, H.P. Bader, R. Scheidegger, H. Singer, A. Lück, I. Hanke, et al., Significance of urban and agricultural land use for biocide and pesticide dynamics in surface waters, Water Res. 44 (2010) 2850–2862. doi:10.1016/j.watres.2010.01.030.

[176] † Akiko Tanabe, † Hideko Mitobe, † Kuniaki Kawata, ‡ and Akio Yasuhara, § Takayuki Shibamoto*, Seasonal and Spatial Studies on Pesticide Residues in Surface Waters of the Shinano River in Japan, (2001). doi:10.1021/JF010025X.

[177] B. Escher, J. Hermens, R. Schwarzenbach, International workshop: Internal exposure - Linking bioavailability to effects, in: Environ. Sci. Pollut. Res., 2005: pp. 57–60. doi:10.1065/espr2005.01.004.

[178] M.S. Greenberg, P.M. Chapman, I.J. Allan, K.A. Anderson, S.E. Apitz, C. Beegan, et al., Passive sampling methods for contaminated sediments: risk assessment and management., Integr. Environ. Assess. Manag. 10 (2014) 224–236. doi:10.1002/ieam.1511.

[179] M. Asgarpour Khansary, S. Shirazian, M. Asadollahzadeh, Polymer-water partition coefficients in polymeric passive samplers, Environ. Sci. Pollut. Res. 24 (2017) 2627–2631. doi:10.1007/s11356-016-8029-7.

[180] M.C. Hennion, Solid-phase extraction: Method development, sorbents, and coupling with liquid chromatography, J. Chromatogr. A. 856 (1999) 3–54. doi:10.1016/S0021-9673(99)00832-8.

[181] A. Andrade-Eiroa, M. Canle, V. Leroy-Cancellieri, V. Cerdà, Solid-phase extraction of organic compounds: A critical review (Part I), TrAC - Trends Anal. Chem. 80 (2016) 641–654. doi:10.1016/j.trac.2015.08.015.

[182] K. Wille, H.F. De Brabander, E. De Wulf, P. Van Caeter, C.R. Janssen, L. Vanhaecke, Coupled chromatographic and mass-spectrometric techniques for the analysis of emerging pollutants in the aquatic environment, Trends Anal. Chem. 35 (2012) 87–108. doi:10.1016/j.trac.2011.12.003.

[183] C.F. Poole, New trends in solid-phase extraction, TrAC - Trends Anal. Chem. 22 (2003) 362–373. doi:10.1016/S0165-9936(03)00605-8.

[184] B. Huerta, A. Jakimska, M. Llorca, A. Ruhí, G. Margoutidis, V. Acuña, et al., Development of an extraction and purification method for the determination of multi-class pharmaceuticals and endocrine disruptors in freshwater invertebrates, Talanta. 132 (2015) 373–381. doi:10.1016/j.talanta.2014.09.017.

[185] C.W. Huck, G.K. Bonn, Recent developments in polymer-based sorbents for solid-phase extraction, J. Chromatogr. A. 885 (2000) 51–72. doi:10.1016/S0021-9673(00)00333-2.

[186] N. Fontanals, R.M. Marcé, F. Borrull, New materials in sorptive extraction techniques for polar compounds, J. Chromatogr. A. 1152 (2007) 14–31. doi:10.1016/J.CHROMA.2006.11.077.

[187] D.W. Brousmiche, J.E. O'Gara, D.P. Walsh, P.J. Lee, P.C. Iraneta, B.C. Trammell, et al., Functionalization of divinylbenzene/N-vinylpyrrolidone copolymer particles: Ion exchangers for solid phase extraction, J. Chromatogr. A. 1191 (2008) 108–117. doi:10.1016/J.CHROMA.2008.01.076.

[188] J.-Y. Pailler, A. Krein, L. Pfister, L. Hoffmann, C. Guignard, Solid phase extraction coupled to liquid chromatography-tandem mass spectrometry analysis of sulfonamides, tetracyclines, analgesics and hormones in surface water and wastewater in Luxembourg, Sci. Total Environ. 407 (2009) 4736–4743. doi:10.1016/J.SCITOTENV.2009.04.042.

[189] W.T. Foreman, J.L. Gray, R.C. Revello, C.E. Lindley, S.A. Losche, L.B. Barber, Determination of Steroid Hormones and Related Compounds in Filtered and Unfiltered Water by Solid-Phase Extraction, Derivatization, and Gas Chromatography with Tandem Mass Spectrometry Techniques and Methods 5-B9, n.d. http://www.usgs.gov/pubprod (accessed December 17, 2018).

[190] E. Vulliet, C. Cren-Olivé, Screening of pharmaceuticals and hormones at the regional scale, in surface and groundwaters intended to human consumption, Environ. Pollut. 159 (2011) 2929–2934. doi:10.1016/j.envpol.2011.04.033.

[191] P. Steven C. Wendelken, Method 539 . 1 : Determination Of Twelve Hormones And Bisphenol A in Drinking Water by Solid Phase Extraction and Liquid Chromatography Electrospray Ionization Tandem Mass Spectrometry, US EPA. (2015). http://water.epa.gov/drink/ (accessed December 11, 2018).

[192] P. Labadie, H. Budzinski, Determination of steroidal hormone profiles along the Jalle d'Eysines River (near Bordeaux, France), Environ. Sci. Technol. 39 (2005) 5113–5120. doi:10.1021/es048443g.

[193] M. Muller, F. Rabenoelina, P. Balaguer, D. Patureau, K. Lemenach, H. Budzinski, et al., Chemical and biological analysis of endocrine-disrupting hormones and estrogenic activity in an advanced sewage treatment plant, Environ. Toxicol. Chem. 27 (2008) 1649–1658. doi:10.1897/07-519.1.

[194] R.A. Trenholm, B.J. Vanderford, J.C. Holady, D.J. Rexing, S.A. Snyder, Broad range analysis of endocrine disruptors and pharmaceuticals using gas chromatography and liquid chromatography tandem mass spectrometry, Chemosphere. 65 (2006) 1990–1998. doi:10.1016/J.CHEMOSPHERE.2006.07.004.

[195] B.L.L. Tan, D.W. Hawker, J.F. M??ller, F.D.L. Leusch, L.A. Tremblay, H.F. Chapman, Comprehensive study of endocrine disrupting compounds using grab and passive sampling at selected wastewater treatment plants in South East Queensland, Australia, Environ. Int. 33 (2007) 654–669. doi:10.1016/j.envint.2007.01.008.

[196] M.L. Etter, J. Eichhorst, D.C. Lehotay, Clinical determination of 17-hydroxyprogesterone in serum by LC-MS/MS: Comparison to Coat-A-Count™ RIA method, J. Chromatogr. B Anal.

Technol. Biomed. Life Sci. 840 (2006) 69–74. doi:10.1016/j.jchromb.2006.04.038.

[197] M.M. Kushnir, A.L. Rockwood, W.L. Roberts, E.G. Pattison, W.E. Owen, A.M. Bunker, et al., Development and performance evaluation of a tandem mass spectrometry assay for 4 adrenal steroids, Clin. Chem. 52 (2006) 1559–1567. doi:10.1373/clinchem.2006.068445.

[198] M.M. Kushnir, A.L. Rockwood, W.L. Roberts, B. Yue, J. Bergquist, A.W. Meikle, Liquid chromatography tandem mass spectrometry for analysis of steroids in clinical laboratories, Clin. Biochem. 44 (2011) 77–88. doi:10.1016/J.CLINBIOCHEM.2010.07.008.

[199] R. Loos, GIVES LEVELS - Analytical Methods for the new proposed Priority Substances of the European Water Framework Directive (WFD), JRC Tech. Rep. (2012) 71. doi:10.2788/51497.

[200] C.J. Weschler, S. Langer, A. Fischer, G. Bekö, J. Toftum, G. Clausen, Squalene and cholesterol in dust from danish homes and daycare centers, Environ. Sci. Technol. 45 (2011) 3872–3879. doi:10.1021/es103894r.

[201] C. Cháfer-Pericás, P. Campíns-Falcó, M.C. Prieto-Blanco, Automatic in-tube SPME and fast liquid chromatography: A cost-effective method for the estimation of dibuthyl and di-2-ethylhexyl phthalates in environmental water samples, Anal. Chim. Acta. 610 (2008) 268–273. doi:10.1016/j.aca.2008.01.040.

[202] A. Gómez-Hens, M.P. Aguilar-Caballos, Social and economic interest in the control of phthalic acid esters, TrAC - Trends Anal. Chem. 22 (2003) 847–857. doi:10.1016/S0165-9936(03)01201-9.

[203] A. Vavrouš, J. Pavloušková, V. Ševčík, K. Vrbík, R. Čabala, Solution for blank and matrix difficulties encountered during phthalate analysis of edible oils by high performance liquid chromatography coupled with tandem mass spectrometry, J. Chromatogr. A. 1456 (2016) 196–204. doi:10.1016/j.chroma.2016.06.014.

[204] Y.Q. Cai, G. Bin Jiang, J.F. Liu, Q.X. Zhou, Multi-walled carbon nanotubes packed cartridge for the solid-phase extraction of several phthalate esters from water samples and their determination by high performance liquid chromatography, Anal. Chim. Acta. 494 (2003) 149–156. doi:10.1016/j.aca.2003.08.006.

[205] L. Herrero, S. Calvarro, M.A. Fernández, J.E. Quintanilla-lópez, M.J. González, B. Gómara, Analytica Chimica Acta Feasibility of ultra-high performance liquid and gas chromatography coupled to mass spectrometry for accurate determination of primary and secondary phthalate metabolites in urine samples, Anal. Chim. Acta. 853 (2015) 625–636. doi:10.1016/j.aca.2014.09.043.

[206] J.W. Munch, DETERMINATION OF PHTHALATE AND ADIPATE ESTERS IN DRINKING WATER\nBY LIQUID-LIQUID EXTRACTION OR LIQUID-SOLID EXTRACTION AND GAS\nCHROMATOGRAPHY WITH PHOTOIONIZATION DETECTION, 1995. https://scholar.google.be/scholar?hl=nl&as_sdt=0%2C5&q=determination+of+phtahlate+and+adipate+esters+in+drinking+water+by+liquid+extraction&btnG= (accessed October 23, 2017).

[207] H. Fromme, T. Küchler, T. Otto, K. Pilz, J. Müller, A. Wenzel, Occurrence of phthalates and bisphenol A and F in the environment, Water Res. 36 (2002) 1429–1438. doi:10.1016/S0043-1354(01)00367-0.

[208] S. Caroli, P. Cescon, D.W.H. Walton, Environmental Contamination in Antarctica: A Challenge to Analytical Chemistry, 2001. https://books.google.be/books?hl=nl&lr=&id=VllXKtrqp5kC&oi=fnd&pg=PR9&dq=environmental+contaminatoion+in+antartic+caroli&ots=7Niqp_sjBd&sig=xDMa1ix2Lbd2qAGRs1SprWPtwSk#v=onepage&q=environmental contaminatoion in antartic caroli&f=false (accessed October 23, 2017).

[209] S. Markager, Practical guidelines for the analysis of seawater, Mar. Biol. Res. 6 (2010) 220. doi:10.1080/17451000903514220.

[210] M.-H. Dévier, K. Le Menach, L. Viglino, L. Di Gioia, P. Lachassagne, H. Budzinski, Ultra-trace analysis of hormones, pharmaceutical substances, alkylphenols and phthalates in two French

natural mineral waters, Sci. Total Environ. 443 (2013) 621–632. doi:10.1016/j.scitotenv.2012.10.015.

[211] G.C.C. Yang, S.H. Liou, C.L. Wang, The influences of storage and further purification on residual concentrations of pharmaceuticals and phthalate esters in drinking water, Water. Air. Soil Pollut. 225 (2014). doi:10.1007/s11270-014-1968-z.

[212] A. Guart, F. Bono-Blay, A. Borrell, S. Lacorte, Effect of bottling and storage on the migration of plastic constituents in Spanish bottled waters, Food Chem. 156 (2014) 73–80. doi:10.1016/j.foodchem.2014.01.075.

[213] D. Amiridou, D. Voutsa, Alkylphenols and phthalates in bottled waters, J. Hazard. Mater. 185 (2011) 281–286. doi:10.1016/j.jhazmat.2010.09.031.

[214] V.G.M. Szilvia Keresztes, Enikő Tatár, Zsuzsanna Czégény, Gyula Záray, Study on the leaching of phthalates from polyethylene terephthalate bottles into mineral water, Sci. Total Environ. 458–460 (2013) 451–458. doi:10.1016/j.scitotenv.2013.04.056.

[215] I. Ustun, S. Sungur, R. Okur, A.T. Sumbul, S. Oktar, N. Yilmaz, et al., Determination of Phthalates Migrating from Plastic Containers into Beverages, Food Anal. Methods. 8 (2015) 222–228. doi:10.1007/s12161-014-9896-5.

[216] M. Greifenstein, D.W. White, A. Stubner, J. Hout, A.J. Whelton, Impact of temperature and storage duration on the chemical and odor quality of military packaged water in polyethylene terephthalate bottles, Sci. Total Environ. 456–457 (2013) 376–383. doi:10.1016/j.scitotenv.2013.03.092.

[217] M.A. Surhio, F.N. Talpur, S.M. Nizamani, M.K. Talpur, H.I. Afridi, A.A. Khaskheli, et al., Leaching of phthalate esters from different drinking stuffs and their subsequent biodegradation, Environ. Sci. Pollut. Res. 24 (2017) 18663–18671. doi:10.1007/s11356-017-9470-y.

[218] T. Górecki, J. Namienik, Passive sampling, TrAC - Trends Anal. Chem. 21 (2002) 276–291. doi:10.1016/S0165-9936(02)00407-7.

[219] M. Shaw, J.F. Mueller, Time integrative passive sampling: How well do chemcatchers integrate fluctuating pollutant concentrations?, Environ. Sci. Technol. 43 (2009) 1443–1448. doi:10.1021/es8021446.

[220] X. Gong, K. Li, C. Wu, L. Wang, H. Sun, Passive sampling for monitoring polar organic pollutants in water by three typical samplers, Trends Environ. Anal. Chem. 17 (2018) 23–33. doi:10.1016/j.teac.2018.01.002.

[221] E.L.M. Vermeirssen, O. Körner, R. Schönenberger, M.J.F. Suter, P. Burkhardt-Holm, Characterization of environmental estrogens in river water using a three pronged approach: Active and passive water sampling and the analysis of accumulated estrogens in the bile of caged fish, Environ. Sci. Technol. 39 (2005) 8191–8198. doi:10.1021/es050818q.

[222] D. a Alvarez, J.D. Petty, J.N. Huckins, T.L. Jones-Lepp, D.T. Getting, J.P. Goddard, et al., Development of a passive, in situ, integrative sampler for hydrophilic organic contaminants in aquatic environments., Environ. Toxicol. Chem. 23 (2004) 1640–1648. doi:10.1897/03-603.

[223] B. Vrana, G.A. Mills, E. Dominiak, R. Greenwood, Calibration of the Chemcatcher passive sampler for the monitoring of priority organic pollutants in water, Environ. Pollut. 142 (2006) 333–343. doi:10.1016/j.envpol.2005.10.033.

[224] C. Harman, I.J. Allan, E.L.M. Vermeirssen, Calibration and use of the polar organic chemical integrative sampler-a critical review, Environ. Toxicol. Chem. 31 (2012) 2724–2738. doi:10.1002/etc.2011.

[225] A.T.K. Tran, R. V. Hyne, P. Doble, Calibration of a Passive Sampling Device for Time-Integrated Sampling of Hydrophilic Herbicides in Aquatic Environments, Environ. Toxicol. Chem. 26 (2007) 435–443.

[226] S.L. Kaserzon, D.W. Hawker, K. Kennedy, M. Bartkow, S. Carter, K. Booij, et al., Characterisation and comparison of the uptake of ionizable and polar pesticides, pharmaceuticals and personal care products by POCIS and Chemcatchers, Environ. Sci. Process. Impacts. 16 (2014) 2517–2526. doi:10.1039/c4em00392f.

[227] J.N. Huckins, J.D. Petty, J.A. Lebo, F. V. Almeida, K. Booij, D.A. Alvarez, et al., Development of the permeability/performance reference compound approach for in situ calibration of semipermeable membrane devices, Environ. Sci. Technol. 36 (2002) 85–91. doi:10.1021/es010991w.

[228] T.L. Ter Laak, F.J.M. Busser, J.L.M. Hermens, Poly(dimethylsiloxane) as passive sampler material for hydrophobic chemicals: Effect of chemical properties and sampler characteristics on partitioning and equilibration times, Anal. Chem. 80 (2008) 3859–3866. doi:10.1021/ac800258j.

[229] S. Endo, S.E. Hale, K.U. Goss, H.P.H. Arp, Equilibrium partition coefficients of diverse polar and nonpolar organic compounds to polyoxymethylene (POM) passive sampling devices, Environ. Sci. Technol. 45 (2011) 10124–10132. doi:10.1021/es202894k.

[230] E.L.M. Vermeirssen, N. Bramaz, J. Hollender, H. Singer, B.I. Escher, Passive sampling combined with ecotoxicological and chemical analysis of pharmaceuticals and biocides - evaluation of three Chemcatcher™ configurations, Water Res. 43 (2009) 903–914. doi:10.1016/j.watres.2008.11.026.

[231] A. Charriau, S. Lissalde, G. Poulier, N. Mazzella, R. Buzier, G. Guibaud, Overview of the Chemcatcher® for the passive sampling of various pollutants in aquatic environments Part A: Principles, calibration, preparation and analysis of the sampler, Talanta. 148 (2016) 556–571. doi:10.1016/J.TALANTA.2015.06.064.

[232] E.L.M. Vermeirssen, C. Dietschweiler, B.I. Escher, J. Van Der Voet, J. Hollender, Transfer kinetics of polar organic compounds over polyethersulfone membranes in the passive samplers pocis and chemcatcher, Environ. Sci. Technol. 46 (2012) 6759–6766. doi:10.1021/es3007854.

[233] M. Bernal-González, C. Durán-Domínguez-de-Bazúa, Development of a Passive Sampler for Monitoring of Carbamate and s-Triazine Pesticides in Surface Waters, Water, Air, Soil Pollut. 223 (2012) 5071–5085. doi:10.1007/s11270-012-1259-5.

[234] M. Kuster, A. De La Cal, E. Eljarrat, M.J. López De Alda, D. Barceló, Evaluation of two aquatic passive sampling configurations for their suitability in the analysis of estrogens in water, Talanta. 83 (2010) 493–499. doi:10.1016/j.talanta.2010.09.033.

[235] A. Vallejo, A. Prieto, M. Moeder, A. Usobiaga, O. Zuloaga, N. Etxebarria, et al., Calibration and field test of the Polar Organic Chemical Integrative Samplers for the determination of 15 endocrine disrupting compounds in wastewater and river water with special focus on performance reference compounds (PRC), Water Res. 47 (2013) 2851–2862. doi:10.1016/j.watres.2013.02.049.

[236] Z. Zhang, A. Hibberd, J.L. Zhou, Analysis of emerging contaminants in sewage effluent and river water: Comparison between spot and passive sampling, Anal. Chim. Acta. 607 (2008) 37–44. doi:10.1016/j.aca.2007.11.024.

[237] X. Shi, J.L. Zhou, H. Zhao, L. Hou, Y. Yang, Application of passive sampling in assessing the occurrence and risk of antibiotics and endocrine disrupting chemicals in the Yangtze Estuary, China, Chemosphere. 111 (2014) 344–351. doi:10.1016/j.chemosphere.2014.03.139.

[238] R. Jacquet, C. Miège, P. Bados, S. Schiavone, M. Coquery, Evaluating the polar organic chemical integrative sampler for the monitoring of beta-blockers and hormones in wastewater treatment plant effluents and receiving surface waters, Environ. Toxicol. Chem. 31 (2012) 279–288. doi:10.1002/etc.737.

[239] A. Arditsoglou, D. Voutsa, Passive sampling of selected endocrine disrupting compounds using polar organic chemical integrative samplers, Environ. Pollut. 156 (2008) 316–324. doi:10.1016/j.envpol.2008.02.007.

[240] O. Posada-ureta, M. Olivares, L. Zatón, A. Delgado, Uptake calibration of polymer-based passive samplers for monitoring priority and emerging organic non-polar pollutants in WWTP effluents, Anal. Bioanal. Chem. (2016) 3165–3175. doi:10.1007/s00216-016-9381-7.

[241] K.A. Maruya, N.G. Dodder, S.B. Weisberg, D. Gregorio, J.S. Bishop, S. Klosterhaus, et al., The Mussel Watch California pilot study on contaminants of emerging concern (CECs): Synthesis

and next steps, Mar. Pollut. Bull. 81 (2014) 355–363. doi:10.1016/j.marpolbul.2013.04.023.
[242] P. Lepom, B. Brown, G. Hanke, R. Loos, P. Quevauviller, J. Wollgast, Needs for reliable analytical methods for monitoring chemical pollutants in surface water under the European Water Framework Directive, J. Chromatogr. A. 1216 (2009) 302–315. doi:10.1016/J.CHROMA.2008.06.017.
[243] M. Petrovic, M. Farré, M.L. de Alda, S. Perez, C. Postigo, M. Köck, et al., Recent trends in the liquid chromatography-mass spectrometry analysis of organic contaminants in environmental samples, J. Chromatogr. A. 1217 (2010) 4004–4017. doi:10.1016/j.chroma.2010.02.059.
[244] P. Vazquez-Roig, C. Blasco, Y. Picó, Advances in the analysis of legal and illegal drugs in the aquatic environment, TrAC - Trends Anal. Chem. 50 (2013) 65–77. doi:10.1016/j.trac.2013.04.008.
[245] D. Barceló, M. Petrovic, Challenges and achievements of LC-MS in environmental analysis: 25 years on, TrAC Trends Anal. Chem. 26 (2007) 2–11. doi:10.1016/J.TRAC.2006.11.006.
[246] F. Hernández, M. Ibáñez, R. Bade, L. Bijlsma, J. V. Sancho, Investigation of pharmaceuticals and illicit drugs in waters by liquid chromatography-high-resolution mass spectrometry, TrAC - Trends Anal. Chem. 63 (2014) 140–157. doi:10.1016/j.trac.2014.08.003.
[247] D.R. Baker, B. Kasprzyk-Hordern, Multi-residue determination of the sorption of illicit drugs and pharmaceuticals to wastewater suspended particulate matter using pressurised liquid extraction, solid phase extraction and liquid chromatography coupled with tandem mass spectrometry, J. Chromatogr. A. 1218 (2011) 7901–7913. doi:10.1016/j.chroma.2011.08.092.
[248] M. Farré, L. Kantiani, M. Petrovic, S. Pérez, D. Barceló, Achievements and future trends in the analysis of emerging organic contaminants in environmental samples by mass spectrometry and bioanalytical techniques, J. Chromatogr. A. 1259 (2012) 86–99. doi:10.1016/j.chroma.2012.07.024.
[249] E. Gracia-Lor, J. V. Sancho, F. Hernández, Multi-class determination of around 50 pharmaceuticals, including 26 antibiotics, in environmental and wastewater samples by ultra-high performance liquid chromatography–tandem mass spectrometry, J. Chromatogr. A. 1218 (2011) 2264–2275. doi:10.1016/J.CHROMA.2011.02.026.
[250] R. Plumb, J. Castro-Perez, J. Granger, I. Beattie, K. Joncour, A. Wright, Ultra-performance liquid chromatography coupled to quadrupole-orthogonal time-of-flight mass spectrometry, Rapid Commun. Mass Spectrom. 18 (2004) 2331–2337. doi:10.1002/rcm.1627.
[251] M. Caban, E. Lis, J. Kumirska, P. Stepnowski, Determination of pharmaceutical residues in drinking water in Poland using a new SPE-GC-MS(SIM) method based on Speedisk extraction disks and DIMETRIS derivatization, Sci. Total Environ. 538 (2015) 402–411. doi:10.1016/J.SCITOTENV.2015.08.076.
[252] D.S. Chormey, Ç. Büyükpınar, F. Turak, O.T. Komesli, S. Bakırdere, Simultaneous determination of selected hormones, endocrine disruptor compounds, and pesticides in water medium at trace levels by GC-MS after dispersive liquid-liquid microextraction, Environ. Monit. Assess. 189 (2017) 277. doi:10.1007/s10661-017-6003-6.
[253] N.H. Torres, M.M. Aguiar, L.F.R. Ferreira, J.H.P. Américo, Â.M. Machado, E.B. Cavalcanti, et al., Detection of hormones in surface and drinking water in Brazil by LC-ESI-MS/MS and ecotoxicological assessment with Daphnia magna, Environ. Monit. Assess. 187 (2015) 379. doi:10.1007/s10661-015-4626-z.
[254] P.B. Fayad, M. Prévost, S. Sauvé, On-line solid-phase extraction coupled to liquid chromatography tandem mass spectrometry optimized for the analysis of steroid hormones in urban wastewaters, Talanta. 115 (2013) 349–360. doi:10.1016/j.talanta.2013.05.038.
[255] S.X.L. Goh, A. Duarah, L. Zhang, S.A. Snyder, H.K. Lee, Online solid phase extraction with liquid chromatography–tandem mass spectrometry for determination of estrogens and glucocorticoids in water, J. Chromatogr. A. 1465 (2016) 9–19. doi:10.1016/j.chroma.2016.08.040.
[256] G. Streck, Chemical and biological analysis of estrogenic, progestagenic and androgenic

steroids in the environment, TrAC - Trends Anal. Chem. 28 (2009) 635–652. doi:10.1016/j.trac.2009.03.006.

[257] L.-P. Zhang, X.-H. Wang, M.-L. Ya, Y.-L. Wu, Y.-Y. Li, Z. Zhang, Levels of endocrine disrupting compounds in South China Sea, Mar. Pollut. Bull. 85 (2014) 628–633. doi:10.1016/j.marpolbul.2013.12.040.

[258] T. Anumol, S.A. Snyder, Rapid analysis of trace organic compounds in water by automated online solid-phase extraction coupled to liquid chromatography–tandem mass spectrometry, Talanta. 132 (2015) 77–86. doi:10.1016/j.talanta.2014.08.011.

[259] A.J. Frey, Q. Wang, C. Busch, D. Feldman, L. Bottalico, C.A. Mesaros, et al., Validation of highly sensitive simultaneous targeted and untargeted analysis of keto-steroids by Girard P derivatization and stable isotope dilution-liquid chromatography-high resolution mass spectrometry, Steroids. 116 (2016) 60–66. doi:10.1016/J.STEROIDS.2016.10.003.

[260] B.C. Moeller, S.D. Stanley, The development and validation of a turbulent flow chromatography-tandem mass spectrometry method for the endogenous steroid profiling of equine serum, J. Chromatogr. B Anal. Technol. Biomed. Life Sci. 905 (2012) 1–9. doi:10.1016/j.jchromb.2012.06.021.

[261] C. Almeida, J.M.F. Nogueira, Determination of steroid sex hormones in water and urine matrices by stir bar sorptive extraction and liquid chromatography with diode array detection, J. Pharm. Biomed. Anal. 41 (2006) 1303–1311. doi:10.1016/J.JPBA.2006.02.037.

[262] E. Ates, K. Mittendorf, H. Senyuva, An automated online turboflowTM cleanup LC/MS/MS method for the determination of 11 plasticizers in beverages and milk, J. AOAC Int. 96 (2013) 1092–1100. doi:10.5740/jaoacint.12-299.

[263] M.A. Soliman, J.A. Pedersen, I.H. Suffet, Rapid gas chromatography-mass spectrometry screening method for human pharmaceuticals, hormones, antioxidants and plasticizers in water, J. Chromatogr. A. 1029 (2004) 223–237. doi:10.1016/j.chroma.2003.11.098.

[264] B. Gómara, R. Lebrón-aguilar, M.J. González, J.E. Quintanilla-lópez, Insight into the retention processes of phthalate metabolites on different liquid chromatography stationary phases for the development of improved separation methods, J. Chromatogr. A. 1423 (2015) 86–95. doi:10.1016/j.chroma.2015.10.069.

[265] New Objective, Technical Note PV-3, Common Background Ions for Electrospray, (2007). http://www.newobjective.com/.

[266] B. (Thermo C. Mahn, LC/MS Contaminant Peaks, 2006. (n.d.). http://www.abrf.org/index.cfm/list.msg/66994.

[267] A. Schlosser, R. Volkmer-Engert, Volatile polydimethylcyclosiloxanes in the ambient laboratory air identified as source of extreme background signals in nanoelectrospray mass spectrometry, J. Mass Spectrom. 38 (2003) 523–525. doi:10.1002/jms.465.

[268] B.O. Keller, J. Sui, A.B. Young, R.M. Whittal, Interferences and contaminants encountered in modern mass spectrometry, Anal. Chim. Acta. 627 (2008) 71–81. doi:10.1016/J.ACA.2008.04.043.

[269] K.M. Verge, G.R. Agnes, Plasticizer contamination from vacuum system O-rings in a quadrupole ion trap mass spectrometer, J. Am. Soc. Mass Spectrom. 13 (2002) 901–905. doi:10.1016/S1044-0305(02)00386-0.

[270] P. Burkhardt-Holm, Linking Water Quality to Human Health and Environment: The Fate of Micropollutants Some of the authors of this publication are also working on these related projects: anthopogenic impacts on cetaceans View project Invasive Fish: Environmental indicators? , 2011. https://www.researchgate.net/publication/268328108 (accessed May 5, 2019).

[271] R. Céspedes, M. Petrovic, D. Raldúa, Ú. Saura, B. Piña, S. Lacorte, et al., Integrated procedure for determination of endocrine-disrupting activity in surface waters and sediments by use of the biological technique recombinant yeast assay and chemical analysis by LC-ESI-MS, Anal. Bioanal. Chem. 378 (2004) 697–708. doi:10.1007/s00216-003-2303-5.

[272] C.P. Feás, M.C.B. Alonso, E. Peña-Vázquez, P.H. Hermelo, P. Bermejo-Barrera, Phthalates determination in physiological saline solutions by HPLC-ES-MS, Talanta. 75 (2008) 1184–1189. doi:10.1016/j.talanta.2008.01.019.

[273] A. Khedr, Optimized extraction method for LC-MS determination of bisphenol A, melamine and di(2-ethylhexyl) phthalate in selected soft drinks, syringes, and milk powder, J. Chromatogr. B Anal. Technol. Biomed. Life Sci. 930 (2013) 98–103. doi:10.1016/j.jchromb.2013.04.040.

[274] J.D. Blair, M.G. Ikonomou, B.C. Kelly, B. Surridge, F.A.P.C. Gobas, Ultra-trace determination of phthalate ester metabolites in seawater, sediments, and biota from an urbanized marine inlet by LC/ESI-MS/MS, Environ. Sci. Technol. 43 (2009) 6262–6268. doi:10.1021/es9013135.

[275] S. Yadav, S. Rai, A.K. Srivastava, S. Panchal, D.K. Patel, V.P. Sharma, et al., Determination of pesticide and phthalate residues in tea by QuEChERS method and their fate in processing, Environ. Sci. Pollut. Res. 24 (2017) 3074–3083. doi:10.1007/s11356-016-7673-2.

[276] I. González-Mariño, R. Rodil, I. Barrio, R. Cela, J.B. Quintana, Wastewater-Based Epidemiology as a New Tool for Estimating Population Exposure to Phthalate Plasticizers, Environ. Sci. Technol. 51 (2017) 3902–3910. doi:10.1021/acs.est.6b05612.

[277] M.C. Barciela-Alonso, N. Otero-Lavandeira, P. Bermejo-Barrera, Solid phase extraction using molecular imprinted polymers for phthalate determination in water and wine samples by HPLC-ESI-MS, Microchem. J. 132 (2017) 233–237. doi:10.1016/j.microc.2017.02.007.

[278] P. López-Roldán, M.J. López De Alda, D. Barceló, Simultaneous determination of selected endocrine disrupters (pesticides, phenols and phthalates) in water by in-field solid-phase extraction (SPE) using the prototype PROFEXS followed by on-line SPE (PROSPEKT) and analysis by liquid chromatography-atmosph, Anal. Bioanal. Chem. 378 (2004) 599–609. doi:10.1007/s00216-003-2187-4.

[279] G.K. Mortensen, K.M. Main, A.M. Andersson, H. Leffers, N.E. Skakkebæk, Determination of phthalate monoesters in human milk, consumer milk, and infant formula by tandem mass spectrometry (LC-MS-MS), Anal. Bioanal. Chem. 382 (2005) 1084–1092. doi:10.1007/s00216-005-3218-0.

[280] L.J. Xu, W. Chu, N. Graham, Sonophotolytic degradation of phthalate acid esters in water and wastewater: Influence of compound properties and degradation mechanisms, J. Hazard. Mater. 288 (2015) 43–50. doi:10.1016/j.jhazmat.2015.02.023.

[281] D. Shah, J. Burgess, A Simple, Fast, and Reliable LC-MS/MS Method for Determination and Quantification of Phthalates in Distilled Beverages, WATERS Appl. Note. (2015) 1–8. http://www.waters.com/webassets/cms/library/docs/720005403en.pdf (accessed March 7, 2018).

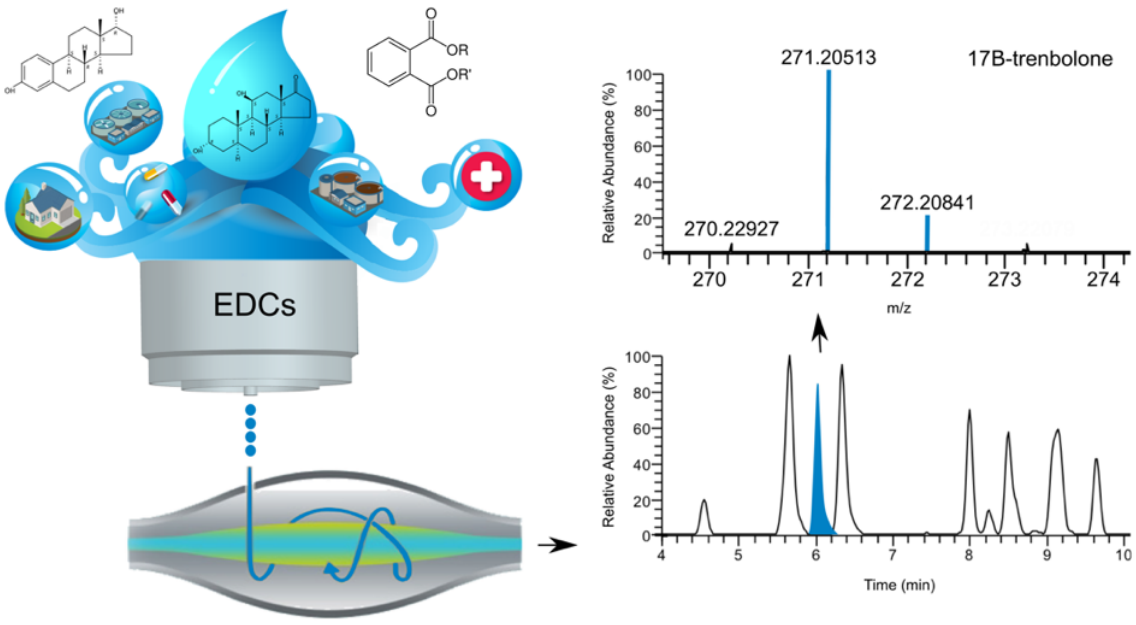
[282] C. Esteve, L. Herrero, B. Gómara, J.E. Quintanilla-López, Fast and simultaneous determination of endocrine disrupting compounds by ultra-high performance liquid chromatography-tandem mass spectrometry, Talanta. 146 (2016) 326–334. doi:10.1016/j.talanta.2015.08.064.

[283] A. Möller, Z. Xie, A. Caba, R. Sturm, R. Ebinghaus, Occurrence and air-seawater exchange of brominated flame retardants and Dechlorane Plus in the North Sea, Atmos. Environ. 46 (2012) 346–353. doi:10.1016/J.ATMOSENV.2011.09.055.

[284] K. Wille, H. Noppe, K. Verheyden, J. Vanden Bussche, E. De Wulf, P. Van Caeter, et al., Validation and application of an LC-MS/MS method for the simultaneous quantification of 13 pharmaceuticals in seawater, Anal. Bioanal. Chem. 397 (2010) 1797–1808. doi:10.1007/s00216-010-3702-z.

II

1 Active sampling



Adapted from:

(1) Huysman S, Van meulebroek L, Vanryckeghem F, Van langenhove H, Demeestere K, Vanhaecke L (2017).
Analytica Chimica Acta 984:140-150

(2) Huysman S, Van meulebroek L, Janssens O, Vanryckeghem F, Van Langenhove H, Demeestere K, Vanhaecke L (2019).
Analytica Chimica Acta 1049:141-151

1. DEVELOPMENT AND VALIDATION OF AN ULTRA-HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC HIGH RESOLUTION Q-ORBITRAP MASS SPECTROMETRIC METHOD FOR THE SIMULTANEOUS DETERMINATION OF STEROIDAL ENDOCRINE DISRUPTING COMPOUNDS IN AQUATIC MATRICES

ABSTRACT

The lack of adequate strategies for monitoring endocrine disrupting compounds (EDCs) in the aquatic environment is emphasized in the European Water Framework Directive. In this context, a new UHPLC-HR-Q-Orbirtrap-MS multi-residue method was developed for the simultaneous measurement of 70 steroidal EDCs in two aquatic matrices, i.e. sea and fresh water. First, an instrumental APCI-UHPLC-HR-Q-Orbitrap-MS was devised for separating and detecting the EDC isomers and mass analogues, within 12.5 min per run. Next, an appropriate extraction was statistically optimised using a three-strep workflow (95% confidence interval, $p > 0.05$); including fractional factorial resolution IV, simplex lattice, and response surface methodological designs. The fitness-for-purpose of the method was demonstrated through successful validation at relevant environmental concentrations, i.e. the low nano- and picogram range. Method quantification limits ranged for the androgens (n = 33), oestrogens (n = 14), progestins (n = 12), and corticosteroids (n = 11) between, respectively, 0.13 and 5.00 ng L^{-1}, 0.25 and 5.00 ng L^{-1}, 0.13 and 2.50 ng L^{-1}, and 0.50 and 5.00 ng L^{-1}. Good linearity ($R^2 \geq 0.99$) and no lack of fit was observed (95% confidence interval, $p > 0.05$) for the 70 steroidal EDCs. In addition, good recovery (95 – 109 %) and satisfactory repeatability (RSD < 8.5 %, n = 18) and reproducibility (RSD < 10.5 %, n = 12) were obtained. Finally, the applicability of the multi-residue method was demonstrated by measuring steroidal EDC in 28 sea water samples collected from four different locations during fall 2016 and winter 2017. Regarding the sea water samples, all the classes were ubiquitously present and included different metabolites, transformation product and or degradation products from the parent EDCs (n = 43).

1.1 INTRODUCTION

The abundance of endocrine disrupting compounds (EDCs) in water is detrimental to the aquatic environment and its ecological health [1,2], resulting in severe consequences such as loss of animal habitats, reduction in biodiversity and intoxication - both acute and chronic - of organisms. For example, in case of fish, such as cyprinids and zebra fish, several negative effects have been reported. Not only do EDCs, such as the synthetic oestrogen 17α-ethinylestradiol or the androgen trenbolone, influence the fertility and reproduction capabilities of fish, they also impact the gender distribution [3–6]**.** In spite of the plethora of adverse effects that have been observed for many EDCs, only 17β-estradiol and 17β-ethinylestradiol have been included in the European watch list for water quality monitoring so far [7]. In order to further improve environmental quality standards, other EDCs, which have received little attention in the aquatic environment, need to be monitored as was recently recommended by Fent et al. (2015) [8].

EDCs mainly include steroidal and non-steroidal compounds. The non-steroidal EDCs comprise contaminants such as phthalates, phenols, antibiotics and polychlorinated biphenyls, whereas the steroidal compound group consists of androgens, oestrogens, progestins, and corticosteroids [9]. Due to the fact that steroidal compounds are the most potent endocrine disruptors in aquatic systems [10,11], these were the main focus within this work. The first steroidal subgroup concerns the androgens, which are the most abundant hormones found in effluents of wastewater treatment plants. These hormones originate from urinary excretion of humans and animals, whereby their presence is due to their usage for therapy and growth treatment [12]. The second steroidal subgroup, i.e. the oestrogens, and in particularly 17α-ethinylestradiol, are widely consumed as oral and non-oral contraceptives [13]. The third steroidal subgroup, i.e. the progestins, are extensively used for contraception and medical treatments and are consumed more than androgens and oestrogens. The last subgroup, i.e. the corticosteroids, are used to treat a variety of diseases, such as asthma, rheumatism, allergies and inflammation [14].

Up to now, studies mainly report on the occurrence of EDCs and related compounds in freshwater environments, whereas data for marine environments are relatively scarce [15,16]. The fresh water environments that have mainly been monitored for EDCs are riverine water, groundwater, drinking water, and wastewater [17–21]. These four major freshwater bodies suffer from contamination primarily due to local anthropogenic activities. As a result, only information on the occurrences of local EDCs is available [22]. As all water eventually ends up in the marine environment and in light of the above-mentioned effects, it is of utter importance to map the contamination status of marine waters as well. However, seawater analysis is complicated by the fact that EDCs prevail in the marine environment in the low ng L^{-1} range and that each EDC can occur in one or more of the following forms: parent EDCs, metabolites, transformation products, and or degradation products [8,23,24]. So far, only three EDCs, namely estrone, 17β-estradiol and 17α-estradiol, have been studied in the marine environment [16].

In this study, a method is presented that allows quantifying 70 target EDCs leaves also the possibility to screen for a virtually unlimited number of (un)known compounds in the marine environment. To realise this, an appropriate extraction and ultra-high performance liquid chromatographic high resolution Q-orbitrap™ mass spectrometric method (UHPLC-HR-Q-orbitrap™-MS) was developed for EDCs in marine waters. The UHPLC-part enables fast simultaneous separation of oestrogens, androgens, progestins and corticosteroids. Furthermore, the HRMS allows a reliable, selective and accurate target detection of the various EDC classes. The analytical method was validated according to CD 2002/657/EC [25], CD 2009/90/EC [26], Eurachem guidelines [27] and review articles [28,29] and eventually applied on real environmental samples.

1.2 MATERIALS AND METHODS

1.2.1 Chemicals and reagents

In this study, 70 steroidal EDCs were included (Table 1 and Table A1), which were purchased at Steraloids Inc (Newport, RI, USA) and Sigma Aldrich (St. Louis, MO, USA). The selected

EDCs were based on relevant literature [15,17–20,30], and covered 4 classes, i.e. 33 androgens, 14 oestrogens, 12 progestins and 11 corticosteroids. The selected deuterated internal standards for each class were purchased at Steraloids (Newport, RI, USA) and Sigma Aldrich (St. Louis, MO, USA) and comprised 6 androgens, 5 oestrogens, 4 progestins, and 2 corticosteroids (Table 1). Primary stock solutions and standard mixtures were prepared in methanol, thereby reaching concentrations between 0.01 and 1000 ng μL^{-1}. The solutions were stored in dark glass bottles at -20°C. The organic solvents were of optima UPLC-MS grade, purchased from Fisher Scientific (Loughborough, UK). Reference seawater was prepared according to ASTM D-1141 [19], using inorganic salts supplied by Sigma Aldrich (St. Louis, MO, USA), i.e. NaCl, Na_2SO_4, MgCl.6(H_2O), $CaCl_2$.2(H_2O), $SrCl_2$.6(H_2O), KCl, $NaHCO_3$, KBr, H_3BO_3 and NaF. Ultrapure water was obtained by usage of a purified-water system (Millipore). The inorganic salts, used to prepare reference seawater, were supplied by Sigma Aldrich (St. Louis, MO, USA). The C_{18} and H_2O-phillic divinylbenzene (DVB) Speedisks were purchased from Filterservice (Eupen, Belgium).

1.2.2 Instrumentation

The EDCs were chromatographically separated using an UHPLC system, consisting of an Ultimate 3000 XRS pumping system, coupled to a Ultimate 3000 RS column compartment and autosampler (Dionex, Amsterdam, The Netherlands). Chromatographic separation was achieved using reversed phase chromatography with gradient elution. Separation of EDCs was carried out using a Hypersil Gold column (1.9 µm, 100 x 2.1 mm) (Intersciences, Louvain-La-Neuve, Belgium) at a temperature of 45°C. Furthermore, the mobile phase consisted of a mixture of water (Eluent A) and methanol (Eluent B) both containing 0.1% formic acid, pumped at a flow rate of 0.55 mL min^{-1}.

Table 1. Instrumental performance of the target compounds included in the multi-EDC UHPLC-APCI-HR-Q-Orbitrap-MS method (mass accuracy was determined through 5 subsequent sample injections). The letters (a-q) in column IS, refer to the internal standard that enabled optimal analytical correction for each steroidal EDC. The internal standards used in this analysis are mentioned at the end of each steroidal EDC class.

Compound	Elemental formula	IS	t_R (min)	Δt_R (min)	Diagnostic ion for quantification	Other diagnostic ions for confirmation	Theoretical mass (*m/z*)	Empirical mass (*m/z*)	Mass Accuracy (Δppm)
Androgens									
Methandriol	$C_{20}H_{32}O_2$	e	6.34	0.01	$[M + H - 2(H_2O)]^+$	$[M + H - (H_2O)]^+$	269.22638	269.22580	0.557
17α-trenbolone	$C_{18}H_{22}O_2$	b	5.52	0.02	$[M + H]^+$		271.16926	271.16867	0.406
17β-trenbolone	$C_{18}H_{22}O_2$	b	4.55	0.03	$[M + H]^+$		271.16926	271.16864	0.479
11β-hydroxyandrosterone	$C_{19}H_{30}O_3$	c	5.65	0.01	$[M + H - 2(H_2O)]^+$	$[M + H]^+$, $[M + H - (H_2O)]^+$	271.20564	271.20498	0.479
Testosterone 17β-cypionate	$C_{27}H_{40}O_3$	d	9.82	0.01	$[M + H - C_8H_{14}O_2]^+$		271.20564	271.20506	0.369
Ethylestrenol	$C_{20}H_{32}O$	e	6.68	0.01	$[M + H - (H_2O)]^+$		271.24203	271.24549	0.811
17β-dihydroandrosterone	$C_{20}H_{34}O_2$	e	6.95	0.01	$[M + H - 2(H_2O)]^+$		271.24203	271.24557	0.442
Androsterone	$C_{19}H_{30}O_2$	e	7.38	0.01	$[M + H - (H_2O)]^+$	$[M + H]^+$	273.22129	273.22058	0.659
Epi-androsterone	$C_{19}H_{30}O_2$	d	6.44	0.01	$[M + H - (H_2O)]^+$	$[M + H]^+$, $[M + H - 2(H_2O)]^+$	273.22129	273.23823	0.915
19-nortestosterone	$C_{18}H_{26}O_2$	b	4.86	0.01	$[M + H]^+$	$[M + H - (H_2O)]^+$	275.20056	275.20005	0.509
3β-androstanediol	$C_{19}H_{32}O_2$	f	8.53	0.01	$[M + H - (H_2O)]^+$		275.23694	275.22389	0.581
3α-androstanediol	$C_{19}H_{32}O_2$	f	8.94	0.01	$[M + H - (H_2O)]^+$		275.23694	275.20038	0.509
1,4-Androstadienedione	$C_{19}H_{24}O_2$	b	3.78	0.02	$[M + H]^+$	$[M + H - (H_2O)]^+$	285.18490	284.51761	0.527
11-ketoetiocholanolone	$C_{19}H_{28}O_3$	b	4.61	0.01	$[M + H - (H_2O)]^+$	$[M + H]^+$	287.20056	287.19993	0.522
Androstenedione	$C_{19}H_{26}O_2$	b	4.84	0.01	$[M + H]^+$	$[M + H - (H_2O)]^+$	287.20056	287.19997	0.627
Mestanolone	$C_{20}H_{32}O_2$	e	7.29	0.01	$[M + H - (H_2O)]^+$	$[M + H]^+$	287.23694	287.23619	0.696
17α-testosterone	$C_{19}H_{28}O_2$	d	6.32	0.01	$[M + H]^+$	$[M + H - (H_2O)]^+$	289.21621	289.21558	0.484
17β-testosterone	$C_{19}H_{28}O_2$	c	5.55	0.01	$[M + H]^+$	$[M + H - (H_2O)]^+$	289.21621	289.21554	0.415
5α-dihydrotestosterone	$C_{19}H_{30}O_2$	e	6.65	0.01	$[M + H]^+$	$[M + H - n(H_2O)]^+$, n=1,2	291.23186	291.23117	0.481
Norethindron	$C_{20}H_{26}O_2$	a	5.15	0.01	$[M + H]^+$	$[M + H - (H_2O)]^+$	299.20056	299.19988	0.401
Methylboldenone	$C_{20}H_{28}O_2$	a	5.23	0.01	$[M + H]^+$	$[M + H - (H_2O)]^+$	301.21621	301.21561	0.764
11-ketotestosterone	$C_{19}H_{26}O_3$	b	3.01	0.01	$[M + H]^+$		303.19547	303.19483	0.561

Compound	Elemental formula	IS	t_R (min)	Δ t_R (min)	Diagnostic ion for quantification	Other diagnostic ions for confirmation	Theoretical mass (*m/z*)	Empirical mass (*m/z*)	Mass Accuracy (Δppm)
Formestano	$C_{19}H_{26}O_3$	b	2.10	0.01	$[M + H]^+$		303.19547	303.19473	0.759
Norethandrolone	$C_{20}H_{30}O_2$	e	7.02	0.01	$[M + H]^+$	$[M + H - (H_2O)]^+$	303.23186	303.23125	0.495
Methyltestosterone	$C_{20}H_{30}O_2$	d	6.22	0.01	$[M + H]^+$	$[M + H - (H_2O)]^+$	303.23186	303.23123	0.429
Trenbolone acetate	$C_{20}H_{24}O_3$	e	7.10	0.01	$[M + H]^+$		313.17982	313.17912	0.543
Ethynyl testosterone	$C_{21}H_{28}O_2$	d	6.24	0.01	$[M + H]^+$		313.21621	313.21551	0.415
Stanozolol	$C_{21}H_{32}N_2O$	e	6.77	0.01	$[M + H]^+$	$[M + H - (H_2O)]^+$	329.25874	329.25798	0.881
Testosterone acetate	$C_{21}H_{30}O_3$	f	8.00	0.01	$[M + H]^+$	$[M + H - C_2H_3O_2]^+$	331.22677	331.22603	0.604
Fluoxymesterone	$C_{20}H_{29}FO_3$	b	4.55	0.01	$[M + H]^+$		337.21735	337.21656	0.623
Testosterone propionate	$C_{22}H_{32}O_3$	f	8.47	0.01	$[M + H]^+$		345.24242	345.24167	0.637
Chlorotestosteron acetate	$C_{21}H_{29}ClO_3$	f	8.25	0.01	$[M + H]^+$	$[M + H - Cl - C_2H_3O_2]^+$	365.18780	365.18699	0.548
Testosterone benzoate	$C_{26}H_{32}O_3$	f	9.05	0.01	$[M + H]^+$		393.24242	393.24153	0.610
Testosterone phenylpropionate	$C_{28}H_{36}O_3$	f	9.14	0.01	$[M + H]^+$	$[M + H - C_7H_8O]^+$	421.27372	421.27264	0.356
19-nortestosterone-17-decanoate	$C_{28}H_{44}O_3$	f	9.99	0.01	$[M + H]^+$	$[M + H - C_{10}H_{19}O_2]^+$	429.33632	429.33525	0.932
17β-trenbolone-d_3 (a)	$C_{18}H_{19}O_2d_3$		5.08	0.01	$[M + H]^+$		273.18181	273.18423	0.549
Nortestosterone-d_2 (b)	$C_{18}H_{24}O_2d_2$		4.86	0.01	$[M + H]^+$		277.21311	277.21256	0.577
17β-testosterone-d_2 (c)	$C_{19}H_{26}O_2d_2$		5.55	0.01	$[M + H]^+$		291.22876	291.22817	0.378
Methyltestosterone-d_3 (d)	$C_{20}H_{27}O_2d_3$		6.22	0.01	$[M + H]^+$		306.25069	306.25003	0.327
Stanozolol-d_3 (e)	$C_{21}H_{29}N_2Od_3$		6.72	0.01	$[M + H]^+$		332.27757	332.27676	0.482
Chlorotestosteron acetate-d_3 (f)	$C_{21}H_{26}ClO_3d_3$		8.22	0.01	$[M + H]^+$		368.20663	368.20574	0.679
Oestrogens									
17α-estradiol	$C_{18}H_{24}O_2$	h	5.23	0.01	$[M + H - (H_2O)]^+$		255.17434	255.17378	0.784
17β-estradiol	$C_{18}H_{24}O_2$	g	4.88	0.01	$[M + H - (H_2O)]^+$		255.17434	255.17397	0.353
Estradiol-17-acetate	$C_{20}H_{26}O_3$	h	7.85	0.01	$[M + H - C_2H_4O_2]^+$		255.17434	255.17366	0.705
Dienoestrol	$C_{18}H_{18}O_2$	h	4.35	0.02	$[M + H]^+$		267.13796	267.13735	0.487
Equilin	$C_{18}H_{20}O_2$	k	4.68	0.01	$[M + H]^+$	$[M + H - (H_2O)]^+$	269.15361	269.15297	0.149
Diethylstilbestrol	$C_{18}H_{20}O_2$	j	4.94	0.01	$[M + H]^+$		269.15361	269.15294	0.706

Compound	Elemental formula	IS	t_R (min)	Δt_R (min)	Diagnostic ion for quantification	Other diagnostic ions for confirmation	Theoretical mass (*m/z*)	Empirical mass (*m/z*)	Mass Accuracy (Δppm)
Estrone	$C_{18}H_{22}O_2$	j	4.94	0.01	$[M + H]^+$	$[M + H - (H_2O)]^+$	271.16926	271.16864	0.627
17α-ethinylestradiol	$C_{20}H_{24}O_2$	j	5.06	0.01	$[M + H - (H_2O)]^+$	$[M + H]^+$	279.17434	279.17368	0.394
α-zearalenol	$C_{18}H_{24}O_5$	j	5.14	0.01	$[M + H - (H_2O)]^+$	$[M + H]^+$, $[M + H - 2(H_2O)]^+$	303.15909	303.15837	0.495
β-zearalenol	$C_{18}H_{24}O_5$	k	4.02	0.01	$[M + H - (H_2O)]^+$	$[M + H]^+$, $[M + H - 2(H_2O)]^+$	303.19090	303.15847	0.429
α-zeranol	$C_{18}H_{26}O_5$	i	4.82	0.01	$[M + H - (H_2O)]^+$	$[M + H]^+$, $[M + H - 2(H_2O)]^+$	305.17474	305.17410	0.524
β-zeranol	$C_{18}H_{26}O_5$	k	3.62	0.01	$[M + H - (H_2O)]^+$	$[M + H]^+$, $[M + H - 2(H_2O)]^+$	305.17474	305.17402	0.492
Gestodene	$C_{21}H_{26}O_2$	j	5.27	0.01	$[M + H]^+$	$[M + H - (H_2O)]^+$	311.20056	311.20034	0.964
Estradiol-benzoate	$C_{25}H_{28}O_3$	h	8.90	0.01	$[M + H]^+$		335.14813	377.21027	0.716
17β-estradiol-d_3 (g)	$C_{18}H_{21}O_2d_3$		4.88	0.01	$[M + H]^+$		277.21036	276.20331	0.688
Dienoestrol-d_2 (h)	$C_{18}H_{16}O_2d_2$		5.84	0.01	$[M + H]^+$		269.15051	269.14971	0.260
Estrone-d_4 (i)	$C_{18}H_{18}O_2d_4$		4.86	0.01	$[M + H]^+$		275.19436	275.20004	0.472
Diethylstilbestrol-d_6 (j)	$C_{18}H_{14}O_2d_6$		4.90	0.01	$[M + H]^+$		275.19127	275.20005	0.509
α-zeranol-d_4 (k)	$C_{18}H_{22}O_5d_4$		4.73	0.01	$[M + H]^+$		327.21041	327.19456	0.092
Progestins									
5α-Pregnan-3α,20β-diol	$C_{21}H_{36}O_2$	o	8.58	0.01	$[M + H - 2(H_2O)]^+$		285.25768	285.25712	0.806
Norgestrel	$C_{21}H_{28}O_2$	m	6.24	0.01	$[M + H]^+$	$[M + H - (H_2O)]^+$	313.21607	313.21552	0.351
Dihydroprogesterone	$C_{21}H_{32}O_2$	m	6.54	0.01	$[M + H]^+$		317.21607	317.21555	0.504
Progesterone	$C_{21}H_{30}O_2$	o	7.24	0.01	$[M + H]^+$		315.23186	315.23148	0.730
Methylprogesterone	$C_{22}H_{32}O_2$	o	8.05	0.01	$[M + H]^+$		329.24751	329.24677	0.547
17α-hydroxyprogesterone	$C_{21}H_{30}O_3$	m	5.77	0.01	$[M + H]^+$	$[M + H - (H_2O)]^+$	331.22678	331.22605	0.483
Megestrol	$C_{22}H_{30}O_3$	m	6.41	0.01	$[M + H]^+$	$[M + H - (H_2O)]^+$	343.22678	343.22599	0.787
Medroxyprogesterone	$C_{22}H_{32}O_3$	m	6.76	0.01	$[M + H]^+$	$[M + H - (H_2O)]^+$	345.24241	345.24163	0.492
17α-acetoxyprogesterone	$C_{23}H_{32}O_4$	m	6.28	0.01	$[M + H]^+$		373.23734	373.23659	0.589
Megestrol acetate	$C_{24}H_{32}O_4$	n	7.03	0.01	$[M + H]^+$		385.23734	385.23648	0.415
Medroxyprogesterone acetate	$C_{24}H_{34}O_4$	l	7.18	0.01	$[M + H]^+$		387.25299	387.25212	0.594
Flugestone acetate	$C_{23}H_{31}FO_5$	m	4.71	0.01	$[M + H]^+$	$[M + H - (H_2O)]^+$	407.22283	407.22203	0.295
Caproxyprogesterone	$C_{27}H_{40}O_4$	o	8.58	0.01	$[M + H]^+$	$[M + H - C_6H_{11}O_2 - C_2H_3O]^+$	429.29994	429.29906	0.629

Compound	Elemental formula	IS	t_R (min)	Δ t_R (min)	Diagnostic ion for quantification	Other diagnostic ions for confirmation	Theoretical mass (*m/z*)	Empirical mass (*m/z*)	Mass Accuracy (Δppm)
Progesterone-d_9 (l)	$C_{21}H_{21}O_2d_9$		7.19	0.01	$[M + H]^+$		324.28835	324.28765	0.648
Megestrol acetate-d_3 (m)	$C_{24}H_{29}O_4d_3$		7.01	0.01	$[M + H]^+$		388.25617	388.25526	0.515
Medroxyprogesterone acetate-d_3 (n)	$C_{24}H_{31}O_4d_3$		7.16	0.01	$[M + H]^+$		390.27182	390.27078	0.692
Melengestrol acetate-d_3 (o)	$C_{25}H_{29}O_4d_3$		7.27	0.01	$[M + H]^+$		400.25617	400.25540	0.550
Corticosteroids									
Prednisone	$C_{21}H_{26}O_5$	p	2.48	0.04	$[M + H - (C_2H_4O_2) - (H_2O)]^+$		299.11972	299.16353	0.100
Corticosterone	$C_{21}H_{30}O_4$	p	3.99	0.01	$[M + H]^+$		347.22169	347.22086	0.461
Cortisone	$C_{21}H_{28}O_5$	p	2.48	0.04	$[M + H]^+$		361.20095	361.19991	0.831
Prednisolone	$C_{21}H_{28}O_5$	p	2.83	0.01	$[M + H]^+$		361.20095	361.19986	0.692
Cortisol	$C_{21}H_{30}O_5$	p	2.83	0.01	$[M + H]^+$		363.21660	363.21566	0.385
Tetrahydrocortisone	$C_{21}H_{32}O_5$	p	2.42	0.04	$[M + H]^+$		365.23225	363.21566	0.275
Corticosterone acetate	$C_{23}H_{32}O_5$	p	5.17	0.02	$[M + H]^+$		389.23225	389.23121	0.668
Dexamethasone	$C_{22}H_{29}FO_5$	q	3.80	0.03	$[M + H]^+$		393.20718	393.20641	0.636
Prednisolone acetate	$C_{23}H_{30}O_6$	p	4.09	0.01	$[M + H]^+$		403.21152	403.21067	0.273
Cortisone acetate	$C_{23}H_{30}O_6$	p	9.05	0.01	$[M + H]^+$		403.21152	403.21057	0.942
Hydrocortisone 21-acetate	$C_{23}H_{32}O_6$	p	4.04	0.01	$[M + H]^+$		405.22717	405.22625	0.642
Prednisone-d_8 (p)	$C_{21}H_{18}O_5d_8$		2.01	0.01	$[M + H]^+$		367.23551	367.23534	0.463
Dexamethasone-d_4 (q)	$C_{22}H_{25}FO_5d_4$		3.76	0.01	$[M + H]^+$		397.23229	397.23143	0.680

The linear gradient program was as follows: 0 min, 40% B; 0-5.8 min, 40-65% B; 5.8-9.0min, 65-100% B; 9.0-10.5 min, 100% B; 10.5-10.6 min, 100-40% B; 10.5-12.5 min, 40% B. The injection volume was 2 µL.

The detection of EDCs was carried out using a Q-Exactive™ Benchtop HRMS (Thermo Fisher Scientific, San-Francisco, USA) fitted with an atmospheric-pressure chemical ionisation (APCI) source. Optimal positive and negative ionisation source working parameters were sheath gas flow 33 a.u. (arbitrary units), auxiliary gas flow 15 a.u., sweep gas flow 2 a.u., discharge current +/- 4 kV, capillary temperature 250°C, and vapour temperature 250°C. The optimal MS parameters of the Q-Exactive™ were S-lens RF-level 70, full-scan events and operated in polarity switching mode. Both scans were performed with a resolution of 70 000 FWHM (Full Width at Half Maximum) at 1 Hz (1 scan per sec) and scan ranges from 60 to 900 Da. Furthermore, the scans were applied by targeting the automatic gain control (AGC) at ultimate mass accuracy (1 x 10^5 ions) and a maximum injection time of 50 ms. Initial instrument calibration was carried out by infusing calibration mixtures for the positive and negative ion mode (LTQ Velos ESI positive and negative ion calibration solution, Thermo Fisher Scientific, San Francisco, USA). Instrument control and data processing were carried out by Xcalibur 4.0 software (Thermo Fisher Scientific, San Francisco, USA).

1.2.3 Sample preparation and extraction

1.2.3.1 Statistical experimental designs for the optimization

A three-step statistical workflow, based on experimental designs, was used to efficiently optimize sample preparation and extraction [32]. For this purpose, reference seawater [31] was used, which was spiked with different amounts of EDCs, according to the optimization stage, i.e. to reach 50 ng L^{-1} for screening (27 experiments); 10 ng L^{-1} for eluent optimization (11 experiments) and 5 ng L^{-1} for response surface modelling (15 experiments).

During the screening phase, 13 parameters that could affect the EDC extraction efficiency were selected based on literature [16,33–35] and investigated for their effects (Table A2). In case of significance, they were retained for further optimization. The significance of these

selected variables was determined by using a three-level fractional factorial resolution IV (n = 27 experiments) experimental design. A second step entailed the optimization of the extraction solvent using a simplex lattice mixture design for three variables (the percentage of methanol, acetonitrile and water) (n = 10 experiments). A third step consisted of optimizing the selected significant variables through response surface modelling (RSM), using a central composited faced-centered (CCF) design (n = 11 experiments).

The software program JMP 12.0 (SAS Institute Inc, Cary, USA) was used to select, evaluate, and model the appropriate statistical experimental designs. All models were optimised using the summarised normalised area, which was selected to take into account the high number of analytes and ensure equal compound contribution. Responses were statistically evaluated by one-way analysis of variance (ANOVA) at a confidence interval of 95% (p-value of 0.05). Finally, the optimised extraction settings that yielded the highest response were calculated by using a generalised reduced gradient non-linear algorithm and RSM.

1.2.3.2 Final protocol for EDC extraction

2.5 L grab samples were filtered (Glass Microfibre Filters Whatman™, 0.45µm, 90 x 90 mm), acidified with 1 M HCl and stored in dark amber glass bottles at 4 °C. Upon extraction, samples were brought to room temperature by vibrating. Thereafter, the pH was adjusted to 7 using 1 M NaOH and a mixture of deuterated internal standards was spiked (n = 17, 25 µL of 10 ng μL^{-1}) to the grab samples. Subsequently, the H_2O-phillic DVB sorbents were conditioned with 20 mL of 5% acetonitrile and 20 mL of ultrapure water under vacuum. Next, the samples were drawn through the H_2O-phillic DVB Speedisks under vacuum, followed by a washing step with 20 mL of ultrapure water, upon which a vacuum was applied on the speedisks to remove residual water drops. Afterwards, elution was performed by gravity using sequential 5 mL of pure acetonitrile and 5 mL of acetonitrile, with the latter being acidified with 0.1% formic acid. The combined extracts were vaporized in the Turbovap under a gentle stream of nitrogen at a temperature of 50°C until dry. After this, the extract was reconstituted in 150 µL of methanol

and ultrapure water (40/60, v/v), centrifuged at 2430 g, and the supernatant was transferred into LC-MS glass vial prior to analysis.

1.2.4 Method validation

The optimised analytical method was validated on reference seawater in order to evaluate its fitness-for-purpose. Currently, no specific criteria for the validation of methods for analysis of micropollutants in the marine environment are available. The only European guideline that is currently available for analytical evaluation of the water status is CD 2009/90/EC [26], in which it is stated that the variation coefficient of the reported concentration must be below 50%. Furthermore, it stipulates that the detection limit has to be 30% below the environmental quality standard, which is defined by the degree of concentration of a substance that water should not exceed to maintain the environmental quality objective. Currently, no environmental quality standards are available concerning the occurrence of steroidal EDCs in the marine environment. Therefore, stricter guidelines were consulted for additional performance criteria in analytical method validation, i.e. CD 2002/657/EC [25], Eurachem guidelines [27] and review articles [28,29].

Evaluation criteria included the empirical method detection (MDL) and quantification limit (MQL), linearity, specificity and selectivity, trueness, and precision. The MDL, MQL and the linearity were investigated by constructing three times a 13-point matrix-matched calibration curve (0, 0.125, 0.25, 0.50, 0.75, 1.0, 2.5, 5.0, 10, 20, 30, 40 and 50 ng L^{-1}). Furthermore, the specificity, selectivity, trueness and precision were examined by spiking the seawater at 1.5, 2.0 and 2.5 times the MQL-level in sixfold. This procedure was repeated on three different days by two different operators. In addition, also 20 blanks, i.e. non-spiked reference seawater, were analysed.

In parallel, a cross-validation on fresh tap water was performed to assess the matrix-versatility of the presented method. During this cross-validation, a 13-point matrix-matched calibration curve was constructed twice to determine the linearity performance, while the specificity,

selectivity, trueness and precision were investigated by enriching the samples with 1.5 times the MQL-level (n = 18).

1.2.5 Application of the analytical method to real samples

The suitability of the method was evaluated by applying it on grab samples, collected at four different locations, i.e. 51.22263°, 2.9357°; 51.340073°, 3.203393°; 51.24683°, 3.113615°; and 51.360494°, 3.113615°, in the Belgian Part of the North Sea (BPNS) during two different periods of the year, i.e. fall 2016 (November 25th) and winter 2017 (February 2th). More specifically, 2.5 L grab samples were taken at a depth of 3 metres, using Niskin bottles [36]. Upon arrival in the lab, samples were acidified to pH 3 using 1 M HCl and stored in dark amber glass bottles at 4°C prior to extraction. Acidification did not significantly impact the recovery of EDCs during extraction optimization. During method optimization, it had been verified that this sample acidification did not affect the recovery of EDCs. Additionally, amber glass bottles were used as storage device. Previous research demonstrated that glass is the best material for storage of aqueous samples for EDC analysis since loss of hydrophobic EDCs was limited to 1 % or less [37,38].

1.3 RESULTS AND DISCUSSION

1.3.1 Method development

1.3.1.1 Liquid chromatography

Given the superior performance of UHPLC in terms of chromatographic resolution compared to conventional HPLC [39], the UHPLC separation strategy was selected in this work for multi-EDC profiling. Optimal conditions, relating to the stationary phase, flow rate, mobile phase composition, additives, column temperature, and injection volume, were determined by studying their impacts on the inter-linked resolution, chromatographic peak shape, and interfering background for the 70 targeted analytes. Optimal UHPLC conditions have been reported earlier (section 2.2.).

Optimization of the UHPLC parameters enabled the separation of 70 target steroidal EDCs (Figures A1 – A6), covering a broad polarity range (log P ranging from 0.5 to 7.9, Table A1) with retention times ranging from 2.1 to 9.9 min (Table 1). In addition, chiral isomers and mass analogues (having overlapping mass extraction windows) were baseline separated, except ethylestrenol and 17β-dihydroandrosterone were separated below the 10% valley rule. This is exemplified in Figure 1, where 8 compounds with an accurate empirical mass of 271.20498 Da and 3 ppm mass tolerance were successfully chromatographically separated (Figure 1), including the chiral compounds 17 α- and β-trenbolone, as well as the mass analogues 11β-hydroxyandrosterone, testosterone acetate, chloro-testosterone acetate, caproxyprogesterone, testosterone phenylpropionate and testosterone 17β-cypionate. Compared to relevant literature [18–20], the overall UHPLC conditions were found to be high throughput for the simultaneous separation of 70 target steroidal EDCs in a single injection with a total run time of 12.5 min.

1.3.1.2 Mass spectrometry

To enable a most reliable and accurate quantification of EDCs, the ionization and mass spectrometric parameters were optimised on seawater extracts. For optimisation of the APCI ionization parameters (reported in section 2.2) the overall peak intensity of the EDCs was the main evaluation criterion. The EDC mass spectra were mainly characterized by the presence of the positive pseudo-molecular ion $[M+H]^+$ and or dehydrated positive pseudo-molecular ion $[M+H-nH_2O]^+$ with n variating between 1 and 2, which has been reported earlier for steroids [40]. The most abundant diagnostic ion was selected for identification and quantitation (Table 1).

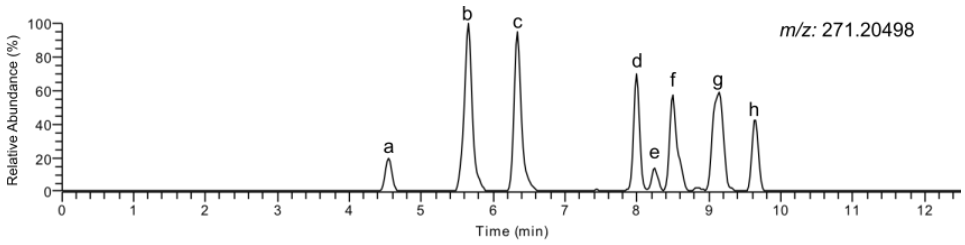


Figure 1. Chromatographic separation of all EDCs with an m/z of 271.20498 Da (mass tolerance = 3 ppm), depicting (a) 17β-trenbolone, (b) 17α-trenbolone, (C) 11β-hydroxyandrosterone, (d) testosterone acetate, (e) chlorotestosterone acetate, (f) caproxyprogesterone, (g) testosterone phenylpropionate and (h) testosterone 17β-cypionate.

The other diagnostic ions were used as an additional confirmation tool backing the isotopic signature of the target compounds, ^{13}C-isotope. The remaining mass spectrometric parameters that were optimized, comprised the resolving power and AGC target. The resolving power was determined by a trade-off between the achievable mass accuracy and the number of data points across the chromatographic peak. On the one hand, an improved mass accuracy (obtained by a higher resolving power) resulted in a better selectivity and thus exclusion of isobaric matrix interferences, which contributes towards unequivocal identification and accurate quantitation. On the other hand, an increasing resolving power was accompanied by less data points across the peak, which resulted in a lack of sensitivity and repeatability. Therefore, a resolving power of 70,000 FWHM was selected since it offered a compromise between high mass accuracy (mass deviations < 3 ppm) and sufficient data points across the chromatographic peaks (> 10) [41]. Furthermore, the optimal AGC target was set to 1e^5 ions, as this setting displayed the lowest mass deviation (< 3 ppm) at the MQL-level.

1.3.1.3 Extraction procedure

A three-level fractional resolution IV experimental design was used for screening, thereby assessing the effect of 13 parameters on the extraction efficiency (Figure 2). Ten parameters turned out to be non-significant (p-value > 0.05) for all EDC classes, whereas the remaining three parameters were observed to be significant (p-value $<$ 0.05) for at least one of the classes. Loading volume was found significant for all classes whereas the solid-phase extraction (SPE) elution steps were significant for some classes. More specifically, the corticosteroids' summarised normalised area was significantly affected by the solvent additive, whereas this was not the case for the elution solvent. However, the elution solvent had a significant effect on the summarised normalised area of the androgens, progestins, and

oestrogens. Based on these findings and the different elution solvents reported in literature [16,33–35], the elution solvent was further optimized using a simplex lattice mixture design, which pointed towards the use of 100% acetonitrile instead of a water-methanol-acetonitrile mixture. The better results that were obtained for acetonitrile are in line with literature, because acetonitrile enables EDCs that are tightly adsorbed to the sorbent phase to undergo lower surface tension/interaction with the sorbent, as such facilitating elution [42]. In a last step, the loading volume and solvent additive were optimised using RSM. Hereby, the RSMs suggested maximal loading volumes.

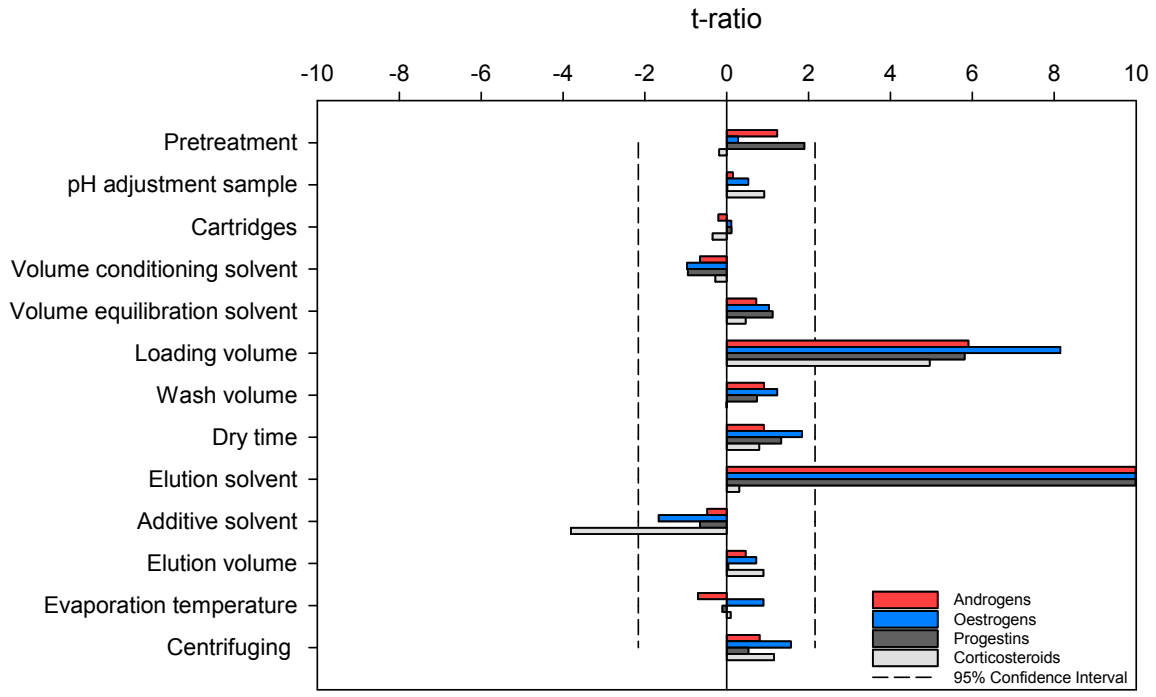


Figure 2. T-ratio (measurement for the size of the difference relative to the variation in the experimental dataset) effect diagram, illustrating the significance of different extraction parameters for the 4 EDC classes on the summarized normalized area. T-ratio effect bars crossing the 95% confidence interval (dashed line) indicate a significant effect of the respective parameter in the extraction process.

Nevertheless, because of clogging of the sorbent phase at this high loading volume, it was technically not possibly to exceed 2.5 L. Optimization of the solvent additive demonstrated a better overall sensitivity for the corticosteroids in the presence of 0.1% formic acid, whereas acid-free solvents favoured the recovery of the androgens, oestrogens and progestins. The use of formic acid is assumed to slightly increase the polarity of acetonitrile, which may result

in a better elution of the more polar compounds including the corticosteroids. Therefore, to efficiently elute the representatives of all EDC classes from the sorbent phase, a sequential use of two elution solvents was implemented; i.e. pure and acidified (0.1% formic acid) acetonitrile.

1.3.2 Method validation

The goal of this work was to develop an analytical methodology that allows the simultaneous quantification of 70 different EDC residues in the marine environment. To ensure accurate quantification, the analytical method was validated, whereby data on the MDL and MQL, specificity, selectivity, linearity, trueness, and precision were generated.

1.3.2.1 Limits of detection and quantification (MDL and MQL)

The detection and quantification of EDC residues using HRMS presents new challenges to the determination of MDLs and MQLs, as traditionally estimated by theoretical or empirical calculations based on signal-to-noise ratios. The signal-to-noise ratios obtained by HRMS are mainly of infinite magnitudes, resulting in virtually low detection and quantification limits. To deal with these virtual estimations, new strategies are required based on more practical criteria. Therefore, the validation criteria stated in CD 2002/657/EC (food safety), CD 2009/90/EC (water monitoring) and Eurachem 2016 (general guidelines) - for measuring residues in the aquatic environment - were combined and refined as was previously described by Vergeynst et al. [43], but with usage of an additional criterion, i.e. identity confirmation through the ^{13}C-isotope and the $^{13}C/^{12}C$-ratio of each target compound at the corresponding theoretical MDL.

The MQLs for the androgens, oestrogens, progestins and corticosteroids ranged from, respectively, 0.13 to 5.00 ng L^{-1}, 0.25 to 5.00 ng L^{-1}, 0.25 to 2.50 ng L^{-1} and 0.50 to 5.00 ng L^{-1}, whereas the MDLs for all classes ranged from 0.06 to 2.50 ng L^{-1}. Our empirical limits, i.e. MDLs and MQLs in seawater (Table 2), are comparable or even lower than the theoretical limits achieved in previous studies, using UHPLC-MS/MS (Table 3).

1.3.2.2 Selectivity

The specificity and selectivity were evaluated by analysing blank samples as well as samples spiked at 1.5 times the MQL (Table A3). As true blanks, reference seawater was used, containing no measurable residues of exogenous EDCs at their accurate mass and specific retention time. A significant increase was observed at the accurate mass and specific retention time when EDCs were added to the blank samples, taking into account a maximal RSD of 20%. The above-mentioned observations confirmed that the optimised method was selective for the 70 target EDCs in the presence of other matrix constituents. Identification was based on accurate mass and relative retention time, i.e. the ratio between retention time of the analyte and its deuterated internal standard, which ensured the high selectivity of the method. In addition, the mass deviation (< 1 ppm) and retention time deviation (< 0.05 min) confirmed the instrumental stability (n = 110, time period = 3 days) of the developed UHPLC-HRMS method (Table 1).

Table 2. Summary of the method validation performance characteristics as determined for EDCs in seawater.

Analyte	Recovery (%)	MDL (ng L^{-1})	MQL (ng L^{-1})	Repeatability RSD (%) (n=18)	Within-laboratory reproducibility RSD (%) (n=12)	R^2	Best IS	Other suitable IS
Androgens								
Methandriol	101.3 ± 6.3	0.06	0.50	6.2 ± 3.0	3.8 ± 1.9	0.9980	e	
17α-trenbolone	100.0 ± 7.2	0.25	0.50	5.5 ± 1.5	6.8 ± 2.8	0.9993	b	m, n
17β-trenbolone	101.6 ± 6.3	0.25	0.50	5.5 ± 1.5	6.8 ± 2.8	0.9949	b	m, n
11β-hydroxyandrosterone	102.5 ± 9.3	0.25	0.50	7.5 ± 4.9	7.9 ± 5.6	0.9949	c	b, f, o
Testosterone 17β-cypionate	108.3 ± 8.4	0.13	0.50	5.8 ± 1.2	8.7 ± 3.2	0.9981	d	b, c, e, f, h
17β-dihydroandrosterone	97.1 ± 6.2	0.50	0.13	6.2 ± 0.8	6.7 ± 0.7	0.9903	e	
Androsterone	100.8 ± 4.8	0.25	0.25	3.7 ± 1.3	4.3 ± 1.4	0.9968	e	a, b, f, m, o, q
19-nortestosterone	97.4 ± 5.6	0.13	0.75	4.8 ± 1.1	6.3 ± 2.5	0.9984	b	c, d, e, h, m
1,4-Androstadienedione	97.4 ± 7.8	0.06	0.25	7.9 ± 0.6	7.1 ± 5.4	0.9987	b	c, d, f, h, l, m, n, o
11-ketoetiocholanolone	98.3 ± 7.8	0.13	0.25	7.0 ± 4.0	7.9 ± 5.3	0.9985	b	i, j
Androstenedione	97.4 ± 5.7	0.13	0.50	4.3 ± 1.5	5.7 ± 0.8	0.9995	b	c, d, f, l, m, n, o, q
Mestanolone	99.8 ± 5.8	0.25	0.75	5.0 ± 0.8	6.6 ± 1.3	0.9965	e	i, j
17α-testosterone	100.3 ± 6.4	0.13	0.25	5.4 ± 2.0	6.3 ± 1.9	0.9975	d	c, e, f, h, l, m, n, o
17β-testosterone	98.9 ± 8.2	0.06	0.25	5.8 ± 3.0	9.6 ± 4.2	0.9998	c	d, e, f, h, l, m, n, o
5α-dihydrotestosterone	98.2 ± 6.3	0.25	0.13	6.4 ± 0.6	6.6 ± 0.7	0.9923	e	
19-Norethindron	98.1 ± 6.3	0.50	1.00	5.0 ± 2.3	7.2 ± 2.7	0.9975	a	i, j
Methylboldenone	100.5 ± 7.9	0.25	1.00	6.9 ± 2.5	7.7 ± 2.6	0.9985	a	b, f
11-ketotestosterone	99.6 ± 8.2	0.13	0.25	6.2 ± 2.9	6.9 ± 3.1	0.9974	b	e
Formestane	100.6 ± 8.6	0.13	0.25	7.8 ± 1.8	8.7 ± 3.1	0.9965	b	c, d, f, h, l, n, o
Norethandrolone	101.8 ± 6.9	0.06	0.13	5.8 ± 2.2	6.8 ± 2.7	0.9962	e	d, l

Analyte	Recovery (%)	MDL (ng L^{-1})	MQL (ng L^{-1})	Repeatability RSD (%) (n=18)	Within-laboratory reproducibility RSD (%) (n=12)	R^2	Best IS	Other suitable IS
Methyltestosterone	98.8 ± 5.7	0.13	0.25	4.8 ± 1.2	6.0 ± 2.3	0.9992	d	c, d, e, f, g, h, l, m, n, o
Trenbolone acetate	100.6 ± 5.8	0.06	0.50	4.7 ± 1.2	5.9 ± 3.1	0.9951	e	a
Ethynyl testosterone	103.3 ± 6.9	0.06	0.25	5.5 ± 2.7	6.0 ± 3.2	0.9987	d	a, b, c, d, e, f, g, h, l, m, n, o, p, q
Stanozolol	98.9 ± 6.6	1.00	1.00	5.3 ± 2.8	8.0 ± 5.7	0.9977	e	b, c, d, f, g, h, l, m, n, p
Testosterone acetate	100.6 ± 6.1	0.06	0.75	4.7 ± 1.4	7.6 ± 2.8	0.9983	f	l
Fluoxymesterone	102.0 ± 6.3	2.50	5.00	5.0 ± 2.0	6.5 ± 1.3	0.9975	b	c, d, g, j, o
Testosterone propionate	100.4 ± 6.4	0.13	0.25	5.1 ± 1.8	7.2 ± 2.4	0.9973	f	b, l, n, o
Chlorotestosteron acetate	100.6 ± 5.6	0.50	0.50	3.9 ± 0.7	7.1 ± 3.0	0.9962	f	a, e
Testosterone benzoate	102.9 ± 6.7	0.50	0.75	4.9 ± 1.9	8.4 ± 3.1	0.9978	f	c, d, l, m
Testosterone phenylpropionate	100.8 ± 6.4	0.25	0.75	5.4 ± 2.2	7.0 ± 2.3	0.9952	f	a, b, c, k, l, n, o, p, q
19-nortestosterone-17-decanoate	102.5 ± 6.7	2.50	2.50	5.4 ± 1.9	7.6 ± 2.3	0.9926	f	h
Oestrogens								
17α-estradiol	101.6 ± 7.8	0.25	5.00	7.0 ± 3.1	7.1 ± 3.1	0.9976	h	a, c, d, i, l, n, o, q
17β-estradiol	100.9 ± 8.4	0.06	2.50	7.0 ± 2.8	8.9 ± 4.6	0.9959	g	a, c, d, h, i, l, n, o, q
Estradiol-17-acetate	100.4 ± 7.8	0.06	0.75	6.7 ± 3.6	8.0 ± 5.3	0.9937	h	c, d, f, l, m, q
Dienoestrol	100.3 ± 7.4	0.25	5.00	6.3 ± 2.9	8.4 ± 5.0	0.9964	h	
Equilin	102.0 ± 7.6	0.13	0.25	6.4 ± 3.2	7.8 ± 4.3	0.9950	k	b, f, m, o
Diethylstilbestrol	101.8 ± 8.3	0.25	0.25	6.2 ± 2.7	10.0 ± 5.7	0.9958	j	b, c, d, f, m, o
Estrone	102.7 ± 8.0	0.06	0.25	6.7 ± 1.8	8.6 ± 3.2	0.9992	j	c, d, n, o
17α-ethinylestradiol	102.8 ± 6.2	2.50	5.00	4.7 ± 1.9	7.8 ± 4.2	0.9958	j	i
a-zearalenol	101.1 ± 6.0	1.00	2.50	6.5 ± 2.7	8.0 ± 4.3	0.9921	j	f
b-zearalenol	101.4 ± 2.9	0.13	0.75	6.0 ± 2.8	7.6 ± 3.7	0.9931	k	h

Analyte	Recovery (%)	MDL (ng L^{-1})	MQL (ng L^{-1})	Repeatability RSD (%) (n=18)	Within-laboratory reproducibility RSD (%) (n=12)	R^2	Best IS	Other suitable IS
a-zeranol	99.9 ± 9.0	0.13	0.75	8.1 ± 2.5	9.7 ± 3.9	0.9947	i	b, d, g, j, l, m, n
b-zeranol	101.4 ± 7.3	0.13	0.75	6.6 ± 1.8	7.0 ± 2.5	0.9908	k	b, d, g, j, l, m, n
Gestodene	103.4 ± 6.6	0.25	0.50	5.1 ± 1.9	7.0 ± 3.5	0.9946	j	b, c, d, e, h, l, m, n, p, q
Estradiol-benzoate	101.2 ± 6.1	1.00	2.50	4.9 ± 0.4	6.9 ± 2.2	0.9941	h	e, l, m
Progestins								
5α-Pregnan-3α,20β-diol	101.3 ± 1.2	2.50	2.50	4.0 ± 1.2	5.8 ± 1.3	0.9917	o	
Norgestrel	101.0 ± 7.1	0.06	0.25	5.3 ± 1.0	6.8 ± 1.2	0.9949	m	a, b, c, d, e, f, g, h, l, m, n, q
Dihydroprogesterone	98.3 ± 8.0	0.06	0.25	6.4 ± 1.1	8.1 ± 2.0	0.9973	m	b, c, d, f, h, i, j, l, n, o
Progesterone	99.0 ± 8.4	0.06	0.50	6.7 ± 4.4	8.8 ± 5.0	0.9984	o	b, c, l, n
Methylprogesterone	102.2 ± 6.2	0.06	0.25	5.5 ± 1.7	5.9 ± 2.1	0.9961	o	a, b, c, d, e, f, h, l, m, n, p
17α-hydroxyprogesterone	100.1 ± 6.9	0.13	0.25	5.7 ± 3.1	6.3 ± 4.1	0.9986	m	b, c, d, f, h, l, n, o
Megestrol	101.9 ± 7.7	0.75	1.00	5.7 ± 2.1	8.8 ± 3.2	0.9966	m	b, c, d, f, h, j, l, n, o
Medroxyprogesterone	101.2 ± 4.5	0.13	0.50	4.1 ± 0.6	4.7 ± 1.1	0.9966	m	b, g, n
17α-acetoxyprogesterone	102.5 ± 5.1	0.13	0.50	4.0 ± 0.6	6.4 ± 2.7	0.9952	m	a, b, c, d, f, l, n, o, q
Megestrol acetate	101.7 ± 5.3	0.50	0.75	4.4 ± 1.2	4.7 ± 1.3	0.9976	n	b, d
Medroxyprogesterone acetate	101.5 ± 3.9	0.50	1.00	3.5 ± 0.4	4.0 ± 0.7	0.9976	l	i, j
Flugestone acetate	102.5 ± 7.2	0.75	1.00	6.2 ± 2.6	6.7 ± 3.1	0.9969	m	b, c, d, e
Caproxyprogesterone	102.0 ± 6.5	0.25	0.75	4.4 ± 0.2	8.5 ± 2.1	0.9977	o	b, c, d, e, f, l, m, n
Corticosteroids								
Prednisone	103.4 ± 7.8	0.25	0.50	6.0 ± 1.8	7.9 ± 1.9	0.9914	p	b, c, d, e, h, m, n
Corticosterone	102.0 ± 5.7	0.50	2.50	5.8 ± 2.4	7.4 ± 3.1	0.9909	p	e
Cortisone	101.7 ± 7.5	0.13	2.50	7.5 ± 3.8	10.5 ± 3.2	0.9952	p	e, l, m

Analyte	Recovery (%)	MDL (ng L^{-1})	MQL (ng L^{-1})	Repeatability RSD (%) (n=18)	Within-laboratory reproducibility RSD (%) (n=12)	R^2	Best IS	Other suitable IS
Prednisolone	102.8 ± 9.1	0.13	2.50	7.0 ± 2.7	8.2 ± 1.6	0.9985	p	
Cortisol	102.3 ± 8.4	0.25	0.75	8.3 ± 5.0	10.0 ± 3.9	0.9926	p	c, i, j
Tetrahydrocortisone	102.5 ± 9.7	0.25	5.00	6.5 ± 2.2	9.3 ± 3.1	0.9985	p	
Corticosterone acetate	103.0 ± 5.7	0.50	2.50	4.2 ± 1.5	5.5 ± 2.6	0.9952	p	a, b, c, d, f, l, j, o
Dexamethasone	103.0 ± 8.5	2.50	2.50	4.2 ± 1.5	5.5 ± 2.6	0.9952	q	
Prednisolone acetate	99.9 ± 6.9	5.00	5.00	5.8 ± 1.4	7.1 ± 2.0	0.9906	p	
Cortisone acetate	101.3 ± 3.2	2.50	2.50	7.8 ± 3.1	7.6 ± 3.8	0.9952	p	
Hydrocortisone 21-acetate	100.0 ± 8.5	5.00	5.00	6.7 ± 1.8	8.7 ± 4.8	0.9984	p	a, c

Table 3. Comparison of our newly developed method with methods from literature for measurement of steroidal EDCs in different aqueous samples.

	This study		**Zhang et al. 2014** [15]		**Torres et al. 2015** [17]		**Petrie et al. 2016** [18]		**Fayad et al. 2013** [19]		**Goh et al. 2016** [20]		**Anumol et al. 2015** [21]	
Matrix	Seawater		Seawater		Surface and drinking water		River water		Wastewater (effluent)		Wastewater		Ultrapure water	
Method	SPE-UHPLC-HRMS		SPE-HPLC-MS/MS		SPE-LC-MS/MS		SPE-UPLC-MS/MS		Online-SPE-LC-MS/MS		Online-SPE-LC-MS/MS		Online-SPE-LC-MS/MS	
Chromatographic column	Hypersil Gold (100 mm x 2.1 mm; 1.9 μm)		Thermo Scientific (100 mm x 3 mm; 3.0 μm)		Zorbax Eclipse Plus (100 mm x 3.0 mm; 3.5 μm)		BEH C18 (150 mm x 1.0 mm; 1.7 μm)		Hypersil Gold (100 mm x 2.1 mm; 1.9 μm)		Accucore (100 mm x 4.6 mm; 2.6 μm)		Agilent Poreshell (50 mm x 2.1 mm; 2.7 μm)	
Ionization	APCI		ESI		ESI		ESI		API		APCI		ESI	
MS device	Q-Exactive Benchtop		MS/MS Agilent Technology		Agilent 6410 triple Quad MS		Xevo TQD triple quadrupole		Quantum Ultra AM triple quadrupole		API 4000™ MS/MS		Agilent 6410 triple Quad MS	
Analysis time (min)	12.5		10		5		22.5		14		17		14.5	
Storage device	Amber glass bottles		Not specified		Amber glass bottles		Amber glass bottles		Amber glass bottles		Amber glass bottles		Amber glass bottles	
Filtration	0.45 μm Whatmann glass filter		0.7 μm GF/F		0.47 μm glass filter		0.7 μm GF/F		0.3 μm glass filter		0.2 μm nylon filter		0.2 μm Captiva PES filter	
SPE sorbent phase	H_2O-phillic DVB		Oasis HLB		Oasis HLB		Oasis MCX		Oasis HLB		Oasis HLB		PLRP	
Sample volume (L)	2.5		8		0.2		0.05		0.01		0.0025		0.0017	
Flow rate (mL min-1)	75		Not specified		4		5		1.5		0.5-1.0		1	
Elution solvent	CH_3CN, 0.1 % CH_2O_2 in CH_3CH		CH_3OH		CH_3OH		0.6 % $C_2H_2O_2$ and 7 % NH_4OH in CH_3OH		CH_3OH		0.1 % NH_4OH in CH_3CN		CH_3CN	
Steroidal compounds	70		4		5		3		8		9		2	
Analytes	MDL (ng L^{-1})	MQL (ng L^{-1})	MDL (ng L^{-1})	MQL (ng L^{-1})	MDL (ng L^{-1})	MQL (ng L^{-1})	MDL (ng L^{-1})	MQL (ng L^{-1})	MDL (ng L^{-1})	MQL (ng L^{-1})	MDL (ng L^{-1})	MQL (ng L^{-1})	MDL (ng L^{-1})	MQL (ng L^{-1})
17β-testosterone	0.06	0.25			0.5	5							2.5	4.4
19-Norethindrone	0.50	1.00							34	35				
17β-estradiol	0.06	2.5	0.1		0.7	7	0.9	4.48	21	36				
Equilin	0.13	0.25									0.44	1.44		
Diethylstilbestrol	0.25	0.25									0.97	3.7		
Estrone	0.06	0.25	0.05		0.5	5	0.78	3.92	16	30	0.16	0.42		
17α-ethinylestradiol	2.50	5.00	0.3		0.7	7	0.98	4.91	18	33	0.6	2.6		
Levonorgestrel	0.06	0.25							18	50			10	11.6
Progesterone	0.06	0.25			0.3	3			8	21				
Cortisone	0.13	2.50									0.48	1.04		
Dexamethasone	2.50	2.50									0.22	0.61		

1.3.2.3 Linearity and deuterated internal standards

Linearity was evaluated by setting up 13-point matrix-matched calibration curves in triplicate, with concentration levels ranging from 0 to 50 ng L^{-1} for the compounds of interest. The linearity was analysed by establishing weighted linear regression models. These regression models (Table A4) indicated good linearity ($R^2 \geq 0.99$) and no lack of fit (95% confidence interval; F-test, $p > 0.05$) [44]. During the evaluation of the linearity performance, appropriate deuterated internal standards were determined for each compound (Table 2), thereby pursuing a RSD for the peak area ratio ≤ 20% and a good linearity ($R^2 > 0.99$ and no lack of fit).

1.3.2.4 Trueness and precision

Trueness and precision were assessed at different levels, which were 1.5, 2.0 and 2.5 times the MQL. In absence of certified reference material, trueness was investigated by calculating the recovery. For all compounds, the recovery ranged between 97% and 109%, with RSDs below 10% (n = 70). These recoveries are better in comparison to literature, ranging in aquatic matrices from 88 to 120% [16,20,45].

The precision, covering the repeatability and within-laboratory reproducibility, was in line with the Horwitz equation. The RSDs of repeatability and within-laboratory reproducibility ranged, respectively, from 3.7 to 8.5% and 3.8 to 10.5% for all compounds (Table 2 and Table A3). These values are comparable to the RSDs that have been described in literature for a rather limited number of EDCs (Table 3), reporting repeatability RDSs from 4.2 to 8.3% and within-laboratory reproducibility RSDs from 3.6 to 12.0% [16,20,45].

1.3.2.5 Cross validation on freshwater

A cross-validation on fresh tap water samples was performed to extend the scope of the method and indicate its versatility (Table A5). Tap water is known for containing free chlorine, and is expected to have different matrix effects [46]. As compared to the performance characteristics obtained for seawater, similar results were achieved for fresh tap water. The

robustness can be explained by the use of matrix-matched calibration curves and multiple suitable deuterated internal standards. Therefore, extending this multi-residue method to more complex aquatic matrices such as the influent and effluent of waste water treatment plants, is anticipated not to drastically alter its performance characteristics.

1.3.3 Application to seawater samples

The suitability of the developed method for target analysis of the 4 EDC subclasses, i.e. androgens, oestrogens, progestins and corticosteroids, was proven through the analysis of 28 seawater samples. The seawater samples were collected from four different locations at the BPNS, during two different sampling campaigns, each with four biological/physical replicates. The average concentrations of the four replicates are depicted in Table 4. The low standard deviations of the four biological/physical replicates confirm the fitness-for-purpose of the developed method for quantifying EDC residues in the marine environment. In addition, the quantified ranges of EDC-residues in the seawater samples confirm the need of applying MQL-levels during validation.

Regarding the multi-EDC profiling analysis, all the classes were ubiquitously present in the seawater samples. Besides the parent EDCs, different metabolites, transformation products, and or degradation products of testosterone, estradiol, and progesterone were quantified (i.e. dihydro, methyl, acetate, propionate, and benzoate form). The most abundant compounds quantified in the seawater samples were the corticosteroids.

Table 4. Detailed data of the grab samples taken at 4 different locations in the BPNS and 2 different time points (for each time point and each location investigated in fourfold, n=4). Blank cells refer to concentrations below the method detection limits.

Grabsamples	Analytical Limits		Sampling Campaign Fall (2016)				Sampling Campaign Winter (2017)		
	MDL	MQL							
	(ng L^{-1})	(ng L^{-1})	51.2263°, 2.9357°	51.2468°, 3.1136°	51.3401°, 3.2003°	51,3605°, 3.1136°	51.2263°, 2.9357°	51.2468°, 3.1136°	51.3401°, 3.2003°
Androgens									
17β-trenbolone	0.25	0.50					0.91 ± 0.47	< MQL	0.57 ± 0.19
11β-hydroxyandrosterone	0.25	0.50		< MQL	0.63 ± 0.08				
Testosterone cypionate	0.13	0.50			1.31 ± 0.35				
Androsterone	0.25	0.25	3.63 ± 0.08	1.61 ± 0.74	3.33 ± 1.43	2.57 ± 0.08		4.41 ± 0.06	
19-nortestosterone	0.13	0.75					< MQL		< MQL
1,4-Androstadienedione	0.06	0.25	1.62 ± 0.30	1.16 ± 0.14	1.48 ± 0.52	1.15 ± 0.30	0.83 ± 0.69	< MQL	0.28 ± 0.07
Androstenedione	0.13	0.50					< MQL	< MQL	< MQL
Mestanolone	0.25	0.75			< MQL			3.34 ± 0.02	3.37 ± 0.01
17α-testosterone	0.13	0.25	1.10 ± 0.26		0.91 ± 0.30	0.51 ± 0.26	0.35 ± 0.07	0.34 ± 0.08	0.26 ± 0.02
5α-dihydrotestosterone			1.89 ± 0.52	0.50 ± 0.17	0.85 ± 0.23	0.52 ± 0.23	< MQL	3.79 ± 0.06	3.79 ± 0.03
11-ketotestosterone	0.13	0.25		1.44 ± 0.10	2.06 ± 0.56		2.31 ± 0.28	0.46 ± 0.03	0.52 ± 0.14
Formestane	0.13	0.25					2.04 ± 0.61	0.83 ± 0.24	0.69 ± 0.09
Methyltestosterone	0.13	0.25		< MQL	0.30 ± 0.07				
Ethynyl testosterone	0.06	0.25	0.65 ± 0.05						0.32 ± 0.01
Testosterone acetate	0.06	0.75	1.12 ± 0.07	0.95 ± 0.03	1.01 ± 0.05	0.96 ± 0.07	< MQL	< MQL	< MQL
Testosterone propionate	0.13	0.25	2.69 ± 1.13	1.24 ± 0.09	1.31 ± 0.12	1.26 ± 1.13		0.48 ± 0.01	0.50 ± 0.02
Testosterone benzoate	0.50	0.75			1.51 ± 0.24				
Oestrogens									
17α-estradiol	0.25	5.00					< MQL	< MQL	< MQL
17β-estradiol	0.06	2.50	9.69 ± 3.96	6.72 ± 0.90	6.40 ± 0.43	6.83 ± 3.96	6.37 ± 0.09		7.62 ± 0.76
Estradiol-17-acetate	0.06	0.75	10.39 ± 6.89	2.83 ± 1.30	3.04 ± 1.71	2.51 ± 0.67		1.49 ± 0.32	2.00 ± 0.73
Dienoestrol	0.25	5.00						< MQL	< MQL
Estrone	0.06	0.25					1.93 ± 0.21	1.90 ± 0.30	1.99 ± 0.46

Grabsamples	Analytical Limits		Sampling Campaign Fall (2016)				Sampling Campaign Winter (2017)		
	MDL	MQL							
	(ng L^{-1})	(ng L^{-1})	51.2263°, 2.9357°	51.2468°, 3.1136°	51.3401°, 3.2003°	51,3605°, 3.1136°	51.2263°, 2.9357°	51.2468°, 3.1136°	51.3401°, 3.2003°
α-zeranol	0.13	0.75					3.85 ± 1.08	3.14 ± 0.42	
Gestodene	0.25	0.50							1.31 ± 0.02
Estradiol-benzoate	1.00	2.50			3.54 ± 0.18				
Progestins									
Norgestrel	0.06	0.25			0.39 ± 0.00				1.73 ± 0.01
Dihydroprogesterone	0.06	0.25						1.92 ± 0.01	1.92 ± 0.01
Progesterone	0.06	0.50	< MQL		< MQL	< MQL		0.81 ± 0.03	0.73 ± 0.06
Methylprogesterone	0.06	0.25	0.75 ± 0.54		< MQL	< MQL	0.66 ± 0.01	0.65 ± 0.02	0.67 ± 0.03
17α-hydroxyprogesterone	0.13	0.25		0.75 ± 0.10				1.78 ± 0.00	1.79 ± 0.02
Megestrol	0.75	1.00			0.41 ± 0.33	1.02 ± 0.33		2.64 ± 0.01	
Medroxyprogesterone	0.13	0.50	0.84 ± 0.33	< MQL	0.65 ± 0.19	0.59 ± 0.09	0.66 ± 0.01	0.65 ± 0.02	0.67 ± 0.03
17α-acetoxyprogesterone	0.13	0.50	1.20 ± 0.11						2.10 ± 0.00
Megestrol acetate	0.50	0.75	< MQL	< MQL	< MQL	< MQL			
Medroxyprogesterone acetate	0.50	1.00	1.14 ± 0.96	< MQL	< MQL	< MQL			
Caproxyprogesterone	0.25	0.75					1.12 ± 0.23		0.89 ± 0.02
Corticosteroids									
Prednisone	0.25	0.50	39.14 ± 8.90	13.09 ± 3.49		9.17 ± 8.90			
Corticosterone	0.50	2.50	4.56 ± 1.73	2.48 ± 0.47	2.59 ± 0.47	3.14 ± 1.73		< MQL	< MQL
Cortisone	0.13	2.50	28.18 ± 17.87	4.79 ± 1.72	6.96 ± 3.23	10.02 ± 17.87	5.51 ± 0.88	4.13 ± 0.31	4.86 ± 0.46
Prednisolone	0.13	2.50		15.17 ± 3.01	6.36 ± 1.49	6.94 ± 1.72	7.71 ± 0.90		6.73 ± 0.24
Cortisol	0.25	0.75	7.48 ± 5.62	0.89 ± 0.98	1.18 ± 0.88	2.81 ± 5.62	3.08 ± 0.15	2.71 ± 0.02	2.72 ± 0.03
Tetrahydrocortisone	0.25	5.00	< MQL	< MQL	< MQL	< MQL	8.77 ± 0.67		
Prednisolone acetate	10.00	10.00	< MQL	< MQL	< MQL	< MQL			

1.4 CONCLUSIONS

A new analytical UHPLC-HR-Q-Orbitrap-MS multi-residue method was developed and successfully validated for the simultaneous quantification of 70 EDCs in sea and fresh water samples. The empirical MQLs in aquatic matrices for the androgens, oestrogens, progestins, and corticosteroids ranged respectively between 0.13 to 5.00 ng L^{-1}, 0.25 to 5.00 ng L^{-1}, 0.13 to 2.50 ng L^{-1}, and 0.50 to 5.00 ng L^{-1}. These low MQLs have shown to be necessary during the environmental application, due to the low concentration levels of EDCs residues.

The newly developed method may constitute an important tool for the holistic monitoring of the EDC contamination of aquatic environments. Moreover, the presented multi-residue method covers the most important EDC classes, and therefore fulfils the current lack of measuring progestins in environmental matrices. This will lead to a better understanding of the ecotoxicological implications of steroidal EDCs in the aquatic environment. Furthermore, the developed method offers the opportunity to screen a virtually unlimited number of (un)known compounds. Finally, monitoring a broad range of EDCs will contribute to the European Water Framework Directive, resulting in better regulations on environmental quality standard levels.

2 TARGETED QUANTIFICATION AND UNTARGETED SCREENING OF ALKYLPHENOLS, BISPHENOL A AND PHTHALATES IN AQUATIC MATRICES USING ULTRA-HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY COUPLED TO HYBRID Q-ORBITRAP MASS SPECTROMETRY

ABSTRACT

Plasticizers and other plastics additives have been extensively used as ingredients of plastics and are as a result thereof easily released in the aquatic environment, due to different physical diffusion processes. In this context, a dedicated method was developed for the simultaneous quantification of 27 known and a virtually unlimited number of unknown alkylphenols, Bisphenol A and phthalates in 2 aquatic matrices, i.e. sea- and freshwater. To this extent, a novel instrumental HESI-UHPLC-HRMS (heated electro-spray ionization ultra-high performance liquid chromatographic high resolution mass spectrometric) method was devised for the simultaneous analysis of 7 phenols (i.e. 6 alkylphenols and Bisphenol A) and 20 phthalates within 10 min. Thereafter, a solid-phase extraction protocol was statistically (95% confidence interval, $p > 0.05$) optimized based on experimental designs. The method was proven fit-for-purpose through a successful validation at environmentally relevant nanomolar concentrations. Analytical precautions were taken for minimizing false-positive results to suppress in-house contamination. The method demonstrated an excellent analytical performance across all known plasticizers and plastics additives for sea- and freshwater, revealing good linearity ($R^2 > 0.99$, $n = 39$), stable recoveries (98.5 - 105.8 %), satisfactory repeatability (RSD < 8%, $n = 54$) and reproducibility (RSD < 10%, $n = 36$). Subsequently, a novel analytical strategy was devised for the tentative identification of unknown plasticizers and plastics additives using specific in-house determined fragments incorporated in a Python code. The applicability of the analytical platform was demonstrated by measuring 24 seawater samples. Interestingly, 16 out of 27 known plasticizers, plastics additives and primary metabolites could be quantified while the untargeted analysis uncovered 1042 compounds,

whereof 5 % (n = 46) could be assigned a plasticizer-plastics additive chemical identity, providing evidence for the severe plastic contamination status of our marine environment.

2.1 INTRODUCTION

The amount of plastic waste that enters our ocean is currently estimated at 4.8 to 12.7 million tons per year. Even more, this cumulative quantity of plastic waste is predicted to increase by an order of magnitude by 2025 [47,48]. As a result of this widespread global contamination of plastics in the marine environment, plasticizers and plastics additives are leaching and inevitable entering marine waters [49]. Indeed, these compounds can be easily released either directly or indirectly, through manufacturing or metabolisation processes as a result of the weak physical bonding with plastic polymers. The most common components that have been used to alter the physical properties of plastics are alkylphenols (APs), bisphenol A, and phthalates (phthalic acid esters, PAEs) [50,51]. APs haven mainly been used in industrial and household applications, covering more than 80% of the total alkylphenolic production [52]. For Bisphenol A, a production of 6.8 billion kg has been reported in 2013 [53]. PAEs are globally synthetized at approximately 6.0 million metric tons per year [54,55]. PAEs are not only used as plasticizer in the polymer industry, but are also used to improve the performance quality of cosmetics, detergents, adhesives, food package materials, personal care products, fragrances, medical devices and lubricants [56,57]. Nowadays, the analysis of APs, Bisphenol A and PAEs in the aquatic environment has received relatively little to almost no attention, especially in comparison to pesticides and pharmaceuticals [58–61]. Nevertheless, the abundance of APs, Bisphenol A and PAEs has recently prompted significant public and mass media interest because of severe known and unknown adverse ecological effects, and possible impact of indirect exposure to human health. For example, bisphenol A causes developmental and reproductive effects in aquatic species, such as zebra fish, frogs and swordtail fish [62,63]. In addition, PAEs can cause severe toxic effects in fish, invertebrates and amphibians [64]. In zebra fish, low doses of diethylhexyl phthalate (DEHP) mainly interfere with steroidogenesis and oocyte growth, while higher doses affect the oocyte maturation [65]. In adults, the disturbance of sex-hormone levels has been observed at low concentrations of

di(2-ethylhexl)phthalate, resulting in severe consequences, including infertility, gynecological disorder, diabetes type 2 and pregnancy-induced hypertension [61].

In spite of the plethora of adverse effects that have been noticed for APs, Bisphenol A, PAEs and their metabolites, only a limited number of these compounds have been included in target lists by regulatory bodies that are responsible for monitoring water quality status, such as the European Watch list, Norman, Reach, Clean Water Act, and OSPAR [7]. In order to further improve environmental quality standards (EQS), it is obvious that an increased number of APs and PAEs should be included in monitoring programs, warranting the requirement of sensitive and reliable analysis methods.

Up until now, studies have mainly reported the occurrence of intact plasticizers and plastics additives in freshwater environments, whereas data for marine environments are rare [66]. Investigated fresh water environments for plasticizer contamination include raw wastewater, groundwater, riverine water, and drinking water [57,67,68]. These four major freshwater bodies receive their contamination load primarily from local anthropogenic activities, resulting in a local contamination profile. To acquire a complete overview of the environmental contamination with plasticizers and plastics additives, it is evident that the marine environment should be monitored as well. Furthermore, metabolite and degradation products should also be included in monitoring strategies, since they display similar biochemical activities as their parent compounds. At present, only a limited number of studies have reported on the determination of phthalate metabolites in the aquatic environment [69,70].

Therefore, this study presents a new analytical platform for simultaneous quantification of 27 known plasticizers, i.e. phenols (n = 7, 6 alkylphenols and Bisphenol A) and PAEs (n = 20, 11 di-phthalates and 9 mono-phthalates) complemented by an untargeted approach for plasticizer metabolite and degradation product detection in the marine environment. To establish this innovative platform, a solid-phase extraction (SPE) and ultra-high-performance liquid chromatographic high-resolution mass spectrometric method (UHPLC-HRMS) were developed and validated for targeted quantification of the selected phenols and PAEs in marine waters. The fitness-for-purpose of this method for marine water monitoring (targeted

and untargeted) was demonstrated by measuring a number of samples originating from the Belgian Part of the North Sea (BPNS). The relevance of samples originating from the BPNS is high, as the latter is located near the English Channel, which is known to be world's busiest seaway and is ranked among the most highly affected marine ecosystems on earth [71,72].

2.2 MATERIALS AND METHODS

2.2.1 Chemicals and reagents

In this study, 27 target compounds were considered (Table 5 and Table B1), which were purchased from Accustandard (New Have, CT, USA) and Sigma Aldrich (St. Louis, MO, USA). The target compounds were selected based on relevant literature, and covered 3 different classes, i.e. 7 phenols, 11 di-phthalates and 9 mono-phthalates [73–75]. The selected internal deuterated standards comprised of 2 phenols, i.e. 2-chlorophenol-d_4 and phenol-d_5; and 2 phthalates, i.e. dicyclohexyl phthalate-3,4,5,6-d_4 and diethyl phthalate-3,4,5,6-d_4. Primary stock solutions and mixed standards, reaching concentrations between 1 and 1000 ng μL^{-1}, were prepared in optima grade acetonitrile. The solutions were stored in amber glass bottles at -20°C. The organic solvents were of optima UHPLC-MS grade, purchased at Fisher Scientific (Loughborough, UK). Reference seawater was prepared according to ASTM D-1141 [31], using inorganic salts supplied by Sigma Aldrich (St. Louis, MO, USA), i.e. NaCl, Na_2SO_4, MgCl.6(H_2O), $CaCl_2$.2(H_2O), $SrCl_2$.6(H_2O), KCl, $NaHCO_3$, KBr, H_3BO_3 and NaF. Ultrapure water was obtained by usage of a purified-water system (Millipore).

2.2.2 Instrumentation

Chromatographic separation of target compounds was executed using a UHPLC system, consisting of an UltiMate 3000 XRS pumping system, coupled to an UltiMate 3000 RS column compartment and autosampler (Dionex, Amsterdam, The Netherlands). Separation of the target compounds was carried out using a Hypersil Gold column (1.9 µm, 100 x 2.1 mm) (Thermo Fisher Scientific, San-Fransisco, USA) at a temperature of 45°C based on gradient

elution. The mobile phase consisted of a mixture of water (Eluent A) and acetonitrile (Eluent B) both containing 0.1% ammonium hydroxide, pumped at a flow rate of 300 µL min^{-1}. The linear gradient program was as follows: 0-1 min, 5 % B; 1-2 min, 5-40 % B; 2-2.3 min, 40-90 % B; 2.3-6.1min, 90-96 % B; 6.1-8 min, 96 % B and 8-10 min, 5 % B. The injection volume was 10 µL. Additionally, a Hypersil Gold trap column (1.9 µm, 50 x 2.1 mm) (Thermo Fisher Scientific, San-Fransisco, USA) was placed between the UHPLC pump and the injection valve for retarding phenols and PAEs originating from the mobile phase and analytical instrument.

The detection of target compounds was carried out using a Q-Exactive™ Benchtop HRMS (Thermo Fisher Scientific, San-Francisco, USA) fitted with a Heated Electrospray Ionization (HESI-II) source. Analysis was realized through full-scan events with following optimal operating conditions for positive and negative ionization (polarity switching mode); auxiliary gas flow 30 arbitary units (a.u.), sweep gas flow 2 a.u., discharge current (-)3.5 kV, capillary temperature 250 °C and heater temperature 350 °C. Optimal MS parameters of the Q-Exactive™ were an S-lens Radio Frequency (RF) level of 70, a resolution of 70,000 FWHM (Full Width at Half Maximum) at 1 Hz, and an m/z scan-range of 60 - 900 Da. Moreover, balanced scans were applied by targeting the automatic gain control (AGC) to $5e^5$ ions and a maximum injection time of 50 ms. Calibration of the instrument was carried out by infusing calibration mixtures for the positive and negative ion mode (LTQ Velos ESI positive and negative ion calibration solution, Thermo Fisher Scientific).

Tentative identification of unknowns, that are related to the backbone of plasticizers and plastics additives, was obtained by combining the full-scan events at a resolution of 70,000 FWHM with an additional Parallel Reaction Monitoring (PRM) HRMS event at a resolution of 17,500 FWHM and optimal Collision Energy (CE) of 20 eV.

Table 5. Summary of the instrumental performance and method validation characteristics for alkylphenol and phthalate analysis with SPE-UHPLC-HR-Q-Orbitrap-MS (MDL=method detection limit (n=39), MQL=method quantification limit (n=39), SD=standard deviation (recovery and repeatability: n=54 and within lab reproducibility: n =18)). The mass spectra of the target compounds were mainly characterized in full-scan as $[M-H]^-$, $[M+H]^+$, and $[M+H]^+$ for phenols, di-phthalates and mono-phthalates, respectively. IS represents the internal standard that was used for correcting variation in the target analyte response.

Compound	Elemental formula	IS	t_r (min)	Accurate mass (*m/z*)	MDL (ng L^{-1})	MQL (ng L^{-1})	Recovery + SD (%)	Repeatability + SD (%)	Within lab reproducibility + SD (%)
Phenols									
2-methyl phenol	C_7H_8O	a	4.99	107.04879	75	100	101.3 ± 3.6	3.6 ± 3.2	6.0 ± 4.7
4-ethylphenol	$C_8H_{10}O$	a	5.08	121.06453	10	25	102.2 ± 3.5	3.4 ± 4.1	4.9 ± 3.0
4-isopropyl phenol	$C_9H_{12}O$	a	5.19	135.08024	150	200	102.5 ± 1.7	1.6 ± 1.3	3.8 ± 2.0
4-chloro-3-methylphenol	C_7H_7ClO	a	5.19	141.01004	10	25	100.4 ± 5.0	4.7 ± 2.0	5.5 ± 1.5
2,5-dichloro phenol	$C_6H_4Cl_2O$	b	1.75	160.95545	25	50	103.7 ± 3.7	3.5 ± 3.8	7.0 ± 3.8
3,4,6-trichlorophenol	$C_6H_3Cl_3O$	a	4.80	194.91693	10	25	104.2 ± 2.6	2.4 ± 1.1	2.4 ± 1.0
Bisphenol A	$C_{15}H_{16}O_2$	a	5.05	227.10737	25	50	101.8 ± 7.8	5.2 ± 3.5	8.0 ± 1.3
Phenol-d_5 (a)	C_6HOd_5		3.77	98.06591					
Chlorophenol-d_4 (b)	C_6HCld_4		1.47	131.01945					
Di-phthalates									
Dimethyl phthalate	$C_{10}H_{10}O_4$	c	5.06	195.06502	25	50	105.8 ± 6.4	6.2 ± 6.8	9.2 ± 2.1
Diethyl phthalate	$C_{12}H_{14}O_4$	c	5.26	223.09621	25	50	105.2 ± 8.1	7.7 ± 2.0	8.9 ± 2.0
Dibutyl phthalate	$C_{16}H_{22}O_4$	c	5.76	279.15863	5	10	101.0 ± 4.6	4.6 ± 2.6	9.9 ± 1.8
Diamyl phthalate	$C_{18}H_{26}O_4$	d	6.12	307.18981	5	25	103.5 ± 9.7	9.5 ± 3.4	9.7 ± 2.2

Compound	Elemental formula	IS	t_r (min)	Accurate mass (*m/z*)	MDL (ng L^{-1})	MQL (ng L^{-1})	Recovery + SD (%)	Repeatability + SD (%)	Within lab reproducibility + SD (%)
Benzyl butyl phthalate	$C_{19}H_{20}O_4$	d	5.70	313.14282	10	25	102.4 ± 8.3	7.7 ± 3.4	9.6 ± 2.0
Dicyclohexyl phthalate	$C_{20}H_{26}O_4$	d	6.18	331.18972	5	20	102.2 ± 5.2	5.1 ± 1.1	6.2 ± 0.5
Dihexyl phthalate	$C_{20}H_{30}O_4$	d	6.51	335.22098	10	20	105.4 ± 7.3	6.9 ± 3.1	7.6 ± 3.2
Dibenzyl phthalate	$C_{22}H_{18}O_4$	d	5.64	347.12718	5	20	103.0 ± 6.8	7.1 ± 1.8	8.6 ± 1.5
Diethylheyxl phthalate	$C_{24}H_{38}O_4$	d	7.52	391.28348	20	25	105.8 ± 1.8	5.8 ± 1.8	7.7 ± 1.7
Dinonyl phthalate	$C_{26}H_{48}O_4$	d	8.00	419.31473	25	25	105.2 ± 5.8	5.8 ± 4.1	7.5 ± 2.6
Diisodecyl phthalate	$C_{32}H_{54}O_4$	d	8.71	447.34688	25	50	102.6 ± 3.9	2.7 ± 2.3	9.3 ± 1.5
Diethyl phthalate-d_4 (c)	$C_{12}H_{10}O_4d_4$		5.05	227.12100					
Dicyclohexyl phthalate-d_4 (d)	$C_{20}H_{22}O_4d_4$		6.26	335.21476					
Mono-phthalates									
Monomethyl phthalate	$C_9H_8O_4$	c	1.00	181.04953	20	25	101.2 ± 6.4	6.2 ± 3.5	9.9 ± 3.3
Monoethyl phthalate	$C_{10}H_{10}O_4$	c	1.02	195.06497	20	25	99.5 ± 5.1	5.1 ± 6.1	5.4 ± 6.6
Monobutyl phthalate	$C_{12}H_{14}O_4$	c	2.35	223.09615	5	25	98.5 ± 1.8	2.5 ± 1.1	3.2 ± 1.6
Mono-n-pentyl phthalate	$C_{13}H_{16}O_4$	d	4.12	237.11174	20	25	102.6 ± 7.9	7.7 ± 5.0	9.7 ± 3.5
Monocyclohexyl phth.	$C_{14}H_{16}O_4$	d	4.04	249.11170	5	10	99.2 ± 7.0	7.0 ± 4.4	7.9 ± 4.9
Monohexyl phthalate	$C_{14}H_{18}O_4$	d	4.24	251.12736	20	25	103.3 ± 5.2	5.0 ± 1.9	7.4 ± 4.4
Monobenzyl phthalate	$C_{15}H_{12}O_4$	d	4.02	257.08033	5	10	98.7 ± 4.4	4.4 ± 2.2	3.4 ± 1.3

Compound	Elemental formula	IS	t_r (min)	Accurate mass (*m/z*)	MDL (ng L^{-1})	MQL (ng L^{-1})	Recovery + SD (%)	Repeatability + SD (%)	Within lab reproducibility + SD (%)
Monoethylhexyl pht.	$C_{16}H_{22}O_4$	d	4.35	279.15879	25	50	101.8 ± 4.9	5.3 ± 2.3	4.5 ± 1.6
Mono-isonyl phthalate	$C_{17}H_{23}O_4$	d	5.30	292.16691	20	25	102.7 ± 3.0	2.9 ± 1.6	5.9 ± 2.8

2.2.3 Sample preparation and extraction

2.2.3.1 Statistical experimental designs for the optimization

A statistical workflow, consisting of 3 experimental designs, was used to efficiently optimise sample preparation and solid-phase extraction (SPE) [66]. First, 14 parameters that could affect the extraction efficiency were selected based on literature (see Table B2) [76–78]. The significant parameters were determined by a three-level fractional factorial resolution IV experimental design (n = 18 experiments) and retained for further optimization. Second the optimal composition of the solid phase eluents was achieved using a simplex lattice mixture design (n = 10 experiments, Table B3) optimizing the percentage of organic solvents, i.e. methanol (CH_3OH), acetonitrile (CH_3CN) and methyl-tert-butylether ($C_5H_{12}O$, MTBE). The more apolar solvent metyl-tert-butylether was tested in a later phase, to assure that adding this solvent would not improve recovery of the target compounds in line with previous work [79,80]. Third, the selected significant parameters were optimised through response surface modelling (RSM), using a box-behnken design (n = 15 experiments). All the experiments were performed using reference seawater that was spiked with 200 ng L^{-1} of each target compound prior to sample preparation and extraction. The above-mentioned experimental designs were selected, evaluated and modelled by JMP 12.0 (SAS Institute Inc, Cary, USA). Moreover, the designs were optimised using the summarized normalized area, thereby acknowledging the high number of analytes and ensuring equal compound contribution. Appropriate designs were selected by maximizing the Chi-efficiency score and minimizing the number of experiments. Thereafter, responses were statistically evaluated by one-way analysis of variance (ANOVA) at a confidence interval of 95% (p-value < 0.05). Finally, optimal extraction settings, yielding the highest response, were calculated using a generalised reduced gradient non-linear algorithm and RSM.

2.2.3.2 Final protocol

Grab samples of 0.5 L were acidified to pH 3 using 1 M HCl and stored in dark amber glass bottles at 4°C. Upon extraction, samples were brought to room temperature. Afterwards, samples were spiked with a mixture of deuterated internal standards, i.e. 100 ng L^{-1} for the deuterated phthalates and 400 ng L^{-1} for the phenols. Subsequently, Oasis® HLB cartridges (6 cc, 500 mg sorbent, 60 µm particle size; Waters) were conditioned with 6 mL 5 % CH3CN diluted in ultrapure water and 7 mL ultrapure water under vacuum. Next, samples were drawn through the cartridges under vacuum (10 mL min^{-1}), followed by a washing step of 8 mL ultrapure water and applying a vacuum (20 min) to remove residual water drops. Afterwards, elution was executed by using 9 mL of 0.1% formic acid in CH_3CN. The extracts were vaporized under a mild stream of nitrogen at a temperature of 40°C until dry. Consequently, the extracts were reconstituted in 150 µL of CH_3CN/H_2O (95/5, v/v), centrifuged at 2430 g. Finally, supernatants were transferred into LC-MS vials prior to analysis.

2.2.4 Method validation

The optimised UHPLC-HRMS method was validated on reference seawater to evaluate its fitness-for-purpose. Currently, there is a lack of specific criteria for validating analytical methods for monitoring organic micropollutants in the marine environment. At the time of execution, the only available European guideline for evaluating the water status was CD 2009/90/EC[26], which stipulates that reported concentrations can have a maximal uncertainty of 50 % or must be below environmental quality standards (EQS). At present, no EQS are available on the abundance of APs, Bisphenol A and PAEs in the aquatic environment, except for DEHP [81]. Detection limits should be 30% below the EQS. Therefore, additional performance criteria in analytical method validation were consulted as stricter guidelines, i.e. CD/2002/657 [44], Eurachem guidelines [27] and review articles [28,29]. The analytical evaluation criteria included the empirical method detection (MDL) and quantification limit (MQL), linearity, specificity and selectivity, trueness, and precision. The MDL, MQL and linearity were examined by establishing a 13-point matrix-matched calibration curve in

threefold at relevant environmental concentrations (0, 5, 10, 20, 25, 50, 75, 100, 200, 400, 600, 800 and 1000 ng L^{-1}). For a limited number of target compounds, i.e. mainly the di-phthalates (and not the mono-phthalates), concentrations were detected up to 1000 ng L^{-1} [73–75]. To evaluate the specificity, selectivity, trueness and precision, seawater was spiked at 1.5, 2.0 and 2.5 times the MQL-level in 6-fold. This procedure was repeated on 3 different days and by 2 operators. Additionally, 20 non-spiked reference seawater samples were analysed as blanks.

A cross-validation on fresh tap water was performed in parallel to assess the matrix-versatility of the presented method. To do this, a 13-point matrix-matched calibration curve was constructed twice to investigate linearity. To evaluate the specificity, selectivity, trueness and precision, the freshwater samples were spiked by 1.5 times the MQL-level (n = 18).

2.2.5 Data analysis

The targeted processing of full-scan data, including identification and quantification of targeted compounds, was executed by XCalibur 4.0 software (Thermo Fisher Scientific). Identification of a compound was realized by use of the accurate mass of the pseudo-molecular parent ion (mass deviation $\leq$ 3 ppm), the C isotope pattern and the retention time relative to that of the internal standard (deviation $\leq$ 2.5%), all being investigated from the corresponding reference standard. Compound Discoverer 2.1 (Thermo Fisher Scientific) was applied for the untargeted data interpretation, characterizing detected ions in terms of accurate mass (*m/z*), retention time, and peak intensity. Parameters for automated peak alignment, noise removal, peak extraction and deconvolution are presented in Table B4. The assignment of characteristic fragments to the untargeted data was processed by an own written code in Python (Version 2.7.), that included the neutral losses and characteristic fragments (mass deviation $\leq$ 3 ppm) as determined for the APs, Bisphenol A and PAEs. During untargeted screening of seawater samples, compounds were tentatively identified using the tier 3 confidence level (according to the Chemical Analysis Working Group & Metabolomics Standards Initiative) [82].

2.2.6 Study area and sampling

The applicability of the SPE followed by UHPLC-HESI-HRMS was demonstrated by quantifying grab samples, collected at four different locations; i.e. 51°21'37.78"N; 3° 6'49.01"O (MOW1), 51°20'25.68"N; 3°12'12.11"O (HZ), 51°14'48.59"N; 2°55'39.61"O (Akust39) and 51°13'34.68"N; 2°56'8.00”O (HO), in the Belgian Part of the North Sea (BPNS) during two different periods of the year, i.e. winter 2016 (November 25th) and spring 2017 (April 10th). A map of the sampling locations can be consulted in Figure A.1. To this end, 0.5 L grab samples were taken in threefold at a depth of 3 m, using Niskin bottles [36]. Upon arrival in the lab, grab samples were acidified to pH 3 using 1 M HCl and stored in dark amber bottles at 4°C prior to extraction.

2.3 RESULTS AND DISCUSSION

2.3.1 Method development

2.3.1.1 Liquid chromatography

GC generally limits the analysis of higher molecular PAEs (ester side-chains containing more than 5 carbons) due to their intermediate volatility (see Table B1). Moreover, time-consuming derivatization steps have shifted the analysis of PAEs to LC in recent years, particularly to UHPLC. UHPLC has been proposed as a superior technique for profiling multiple phenols and PAES as compared to conventional HPLC [83]. In general, UHPLC offers a better resolution (5 - fold), speed (10 - fold), sensitivity (analyte specific) and reduced solvent consumption (5-fold) for analytical determinations as opposed to HPLC [83,84]. Therefore, UHPLC was the platform of choice for targeting a broad range of low and high molecular PAEs. UHPLC separation methods for PAEs are however scarce [85], and has already been proven to be very challenging for AP analysis (because of their high volatility - see Table B1). Hence, the optimization of the UHPLC conditions - including stationary phase, flow rate, mobile phase composition, additives, column temperature, and injection volume - were studied in detail by

evaluating the inter-linked resolution ($R_{s,minimal}$ = 0.28 and $R_{s,opitmal}$ = 10.00), chromatographically symmetric peak shape ($A_{s,minimal}$ = 1.50 and $A_{s,optimal}$ = 1.00) and potential interfering background of the 27 target analytes. Moreover, interfering background peaks of diethyl hexyl and dinonyl phthalate were observed in almost every analytical run (Figure 3). Figure 3 depicts the varying area (intensity) of the interfering background peaks (uncoloured area of Figure 4) of diethyl hexyl and dinonyl phthalate. Figure 3 depicts the fluctuating area (intensity) of the interfering background peaks (not coloured area of Figure 4) of the of diethyl hexyl and dinonyl phthalate. Therefore, a number of analytical precautions were taken to minimize false positive results and favour reliable quantification. First, a trap column was placed between the UHPLC pump and the injection valve for retarding any PAEs and phenol contaminations originating from the analytical instrument and eluent. This is exemplified in Figure 4, representing the chromatographic delay of the interfering background peaks (uncoulored area in Figure 4) of diethyl hexyl and dinonyl phthalate as compared to the target analytes (colored area in Figure 4).” Without the use of this trap column, the varying background contamination (depicted in Figure 3 for diethyl hexyl and dinonyl phthalate) of the analytical instrument and eluent would interfere with the analysis of the compounds of interest originating from the samples. Second, the eluent acetonitrile - instead of the conventional methanol - was selected to minimize transesterification of target and untargeted PAEs into the primary mono-methyl phthalate. Controlling the degree of transesterification results in better quantification of target PAEs and identification of untargeted PAEs. Indeed, this transesterification has been observed when methanol was combined with formic acid at the high prevailing temperatures and voltages of the ionization source [86]. Furthermore, instability of the retention times (within a retention time window of 1.5 min) were observed for the APs using formic acid because the pH of the mobile phase was near the pKa of the compounds. Therefore, ammonium hydroxide was selected as mobile phase additive, having the supplementary benefit of an enhanced ionization rate for the alkylphenols. The results of UHPLC optimization can be consulted for the standards, blanks and spiked samples in Figures B2 – B10.

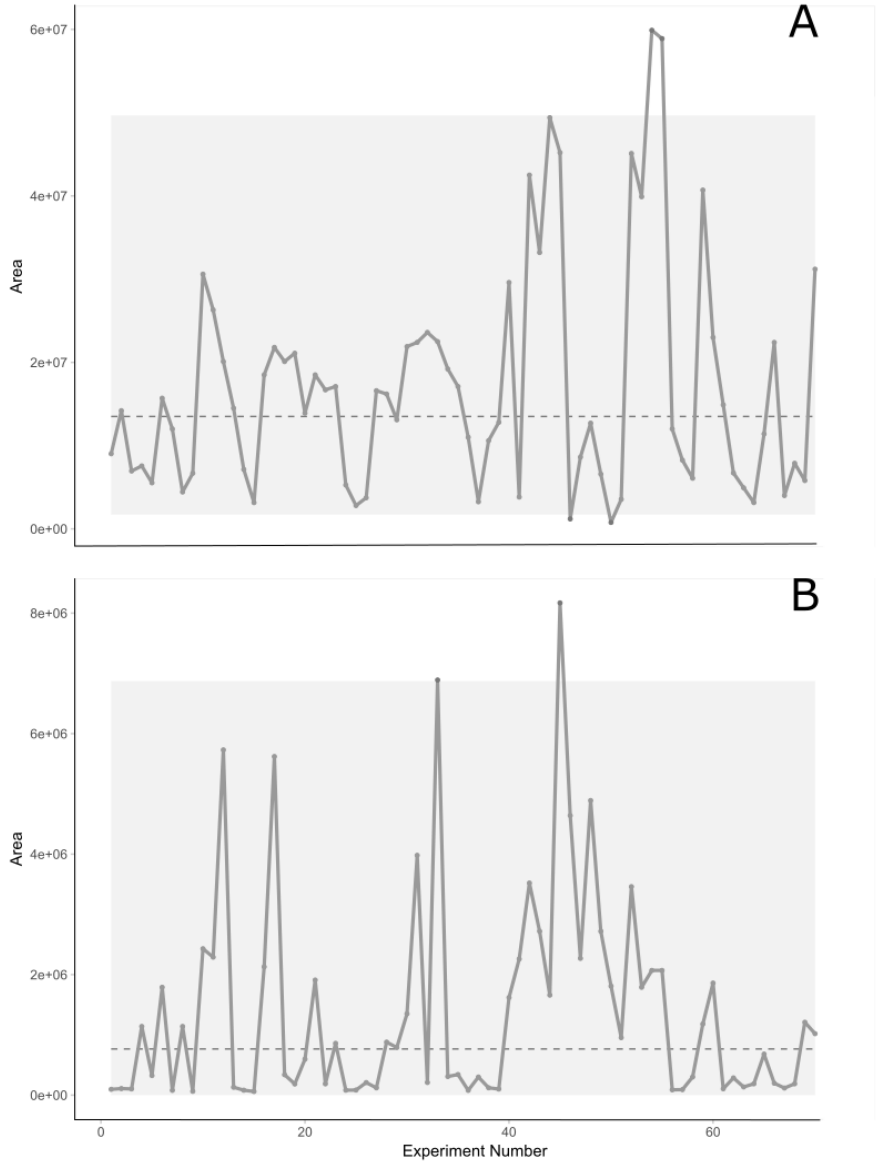


Figure 3. Control chart of the interfering background that was delayed by using a trap column, i.e. (a) di-ethyl hexyl phthalate and (b) dinonyl phthalate. The dotted line represents the mean, and the grey shaded area represents the area between the upper and lower central limit.

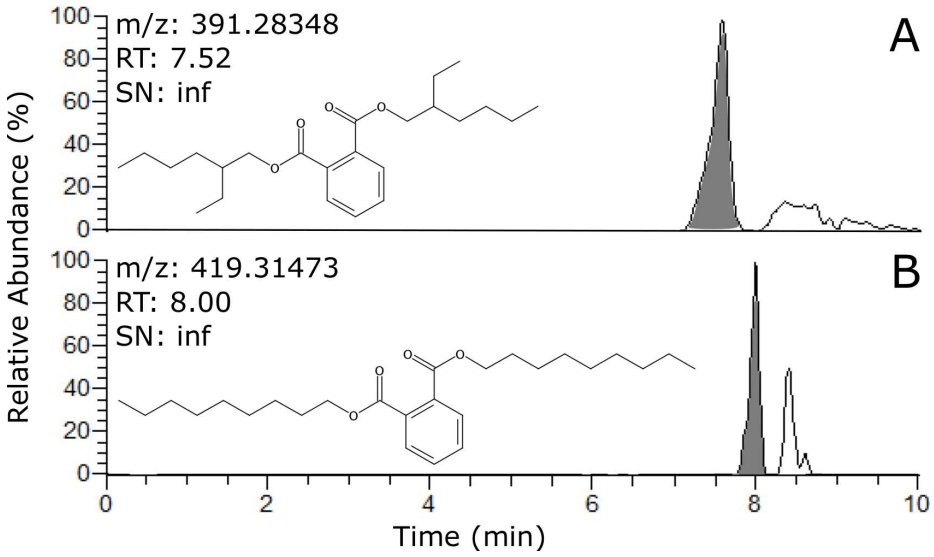


Figure 4. UHPLC-HRMS chromatograms of the target compounds (coloured) that are separated from the interfering background (not coloured), i.e. (a) di-ethyl hexyl phthalate and (b) dinonyl phthalate.

2.3.1.2 Ionization and full-scan mass spectrometry

Reliable and accurate quantification was achieved by optimizing the HESI and HRMS conditions upon evaluation of the overall peak intensity of the target compounds. The specific suspected pseudo-molecular ions for a salt matrix were not observed in full-scan, i.e. adducts of [Na]+, [K]+ and [NH4]+ [87]. Instead, the mass spectra of the target compounds were mainly characterized in full-scan as [M+H]+, [M+H]+ and [M-H]- for di-phthalates, mono-phthalates and phenols, respectively. The abundant pseudo-molecular ions and their corresponding 13C-isotope were selected for accurate identification and quantification (Table 5). Remaining full-scan MS parameters, i.e. the resolving power and AGC target, were optimized. The resolving power was determined by optimizing the balance between sufficient number of data points across the chromatographic peak and a minimal mass deviation. Improving the mass accuracy (achieved by a higher resolving power) resulted in a better selectivity and consequently exclusion of isobaric matrix interferences, which contributed towards unambiguous identification and accurate quantitation. However, increasing the resolving power also resulted in less data points across the chromatographic peak, which negatively affected the repeatability and sensitivity. Therefore, a resolving power of 70,000 FWHM was retained to

acquire sufficient data points across the chromatographic peak (> 10) but at the same time accommodate sufficiently high mass accuracies (mass deviations < 3 ppm) [41]. The optimal AGC target was set at 5e5 ions, as this setting demonstrated the lowest mass deviation (< 3 ppm) at MQL-level.

2.3.1.3 Extraction procedure

Preliminary experiments (See Table B4) demonstrated that 2 of the 11 commercially available SPE cartridges were appropriate for target compound clean up (i.e. Oasis™ HLB and Strata X™, based on the highest recovery, number of analytes, best reproducibility and lowest contamination in the blanks). These 2 cartridges were retained for the first step of the three-step statistical workflow for optimizing the phenol and PAE extraction procedure.

As described in the material and methods, first, the statistical significance of 14 extraction parameters on the phenols (i.e. AP and Bisphenol A) and PAE (i.e. mono- and di-phthalates) recovery was determined using a three-level fractional factorial resolution IV experimental design (Table B5). Nine parameters were significant (p-value < 0.05) for the di-phthalates, whereas for the mono-phthalates and phenols, respectively, 2 and 3 parameters were found significant. Significant parameters included filter step, pH, type of cartridge, volume of the equilibration solvent, loading volume, wash volume, elution solvent, elution solvent additive, volume of the elution solvent and evaporation temperature. The individual significance can be consulted in Table B5. After the screening phase, the following significant parameters were fixed: pre-treatment, type of cartridge, conditioning solvent, and additive (based on the optima) and pH, loading volume and evaporation temperature (based on optima and technical limitations). The other significant parameters were optimized in later steps.

Based on our initial findings and different elution solvents reported in literature [79,88], the elution solvent was further optimized in a 2nd step using a simplex lattice mixture design, which pointed towards the use of 100 % CH_3CN instead of a MTBE-methanol-acetonitrile mixture (Figure B.11). This is in line with literature, as CH_3CN facilitates the elution of PAEs tightly adsorbed to the sorbent phase by undergoing lower surface tension/interactions with the

sorbent [54]. Recently Jeong et al. [89] demonstrated that the surface tension/interaction of organic compounds to Oasis HLB™ in aquatic matrices is mainly dominated by physisorption and enhanced by chemisorption. Chemisorption is mainly driven by π-π interactions between the sorbent and the target compounds, due to the aromatic structure of the target compounds. This π-π interaction is however impeded when using acetonitrile [90].

In the last step, the equilibration, wash and elution volume of solvent were optimized using RSM (Figure B.12) for providing maximal extraction efficiency. The final and optimized extraction procedures are reported above.

2.3.1.4 Analytical precautions

The use of plastic as glass-substitute was tested for the potential contamination with PAEs, as recommended in literature [86]. Therefore, a home-made database of 51 PAEs was used (Table B7), including potential contaminants that have been reported during quantitative analysis [51,91]. No significant difference ($p > 0.05$) was observed between glass and polypropylene micropipette tips, and none of the contaminants from the home-made database were detected. A significant loss ($p < 0.05$) of high molecular PAEs, i.e. diamyl, benzyl butyl, dibenzyl, and diisodecyl phthalate, was however observed during the evaporation of the eluent in glass, which was not the case for polypropylene falcon tubes (TPP, Switzerland). Prospectively, the polypropylene falcon tubes were selected as material of choice.

2.3.2 Method validation

2.3.2.1 Limits of detection and quantification (MDL and MQL)

Determining MDLs and MQLs of target analytes when using HRMS gives rise to new challenges. Traditionally, MDLs and MQLs are estimated by theoretical or empirical calculations based on signal-to-noise ratios. Signal-to-noise ratios are, however, often of infinite magnitude when using HRMS, resulting in virtually infinitely low MDLs and MQLs. These unrealistic estimations stress the need of new strategies based on more practical criteria. Therefore, validation criteria for measuring emerging micropollutants in the aquatic

environment were combined and refined, i.e. CD 2002/657/EC (food safety), CD 2009/90/EC (water monitoring) and Eurachem 2016 (general guidelines) as previously described by Vergeynst et al. [43]. In brief, the MDL was determined using a multi-injection statistical methodology commonly applied for trace analysis. Using the mean value and standard deviation of biological replicate extractions provides a statistically valid approach to discriminate the differences between a low-level analyte (near MDL) and the combined uncertainties in both the analyte and background measurements, and the uncertainty in the sampling process. The MDLs, determined by using the latter statistical tool, were practically confirmed by spiking reference seawater at MDL level. Thereby, an additional confirmation criteria was used for approving the reliability of the MDL, i.e. the presence of the ^{13}C-isotope and $^{13}C/^{12}C$-ratio of each target compound at the concentration investigated. The presence of the ^{13}C-isotope and C^{13}/C^{12} ratio has been frequently used as an additional confirmatory tool in omics studies for enhancing analytical accuracy [92]. Moreover, as long as the ^{13}C-isotope with the corresponding ^{12}C-isotope of the target compound is detectable, the presence of the compound can be undoubtedly confirmed. If the ^{13}C-isotope is no longer detectable, the presence and identity of the target analyte is questionable. The latter criterium was also used to fine tune the MDLs. Furthermore, the determination of the $^{13}C/^{12}C$-ratio also enables to determine the number of carbon atoms present in the target compound. As a consequence, matching experimental and theoretical number of carbon atoms of the target compound provides sufficient evidence for its presence in aquatic samples and the reliability of the MDLs [92]. The MQL on the other hand is regarded as the smallest quantity of a target compound that can be detected in a sample with an RSD below 20% of at least 3 independent measurements using spiked reference blank samples. This 20% criterium has been indicated in many regulations (such as 2002/657/EC, 2009/90/EC and CD 2013/39/EU) as the maximal allowed variation that can been considered as reliable [7,26,44]. Ultimately, considering the above-mentioned approaches, the MDLs for phenols, di-phthalates and mono-phthalates ranged respectively from 10 to 150 ng L-1, 5 to 25 ng L^{-1} and 5 to 25 ng L^{-1}, whereas the MQLs ranged respectively from 25 to 200 ng L^{-1}, 10 to 50 ng L^{-1} and 10 to 50 ng L^{-1}. The MQLs

attained are sufficiently low, based on the only available EQS in literature for DEHP, i.e. 1.3 µg L^{-1} in surface waters [93].

2.3.2.1.1 Specificity and Selectivity

No detectable residues of exogenous APs, Bisphenol A and PAEs at their accurate mass and specific retention time were observed in reference seawater used as a blank (Table 5 and, Figures B5 - B10). Similar conductivity and salinity were noticed between reference and real seawater, which can be consulted in Table B8. Spiking the target analytes to the blanks resulted in a significant increase, taking into account a maximal RSD of 20% (Table B9), confirming the selectivity of the optimised method for the 27 target compounds. The latter were identified based on their accurate mass and relative retention time, i.e. the ratio between retention time of the analyte and its deuterated internal standard. Moreover, the target low and high molecular phthalates were respectively corrected by using the diethyl phthalate-3,4,5,6-d_4 and dicyclohexyl phthalate-3,4,5,6-d4. The specific deuterated internal standard that was used for quantification of every target compound can be consulted in Table 5. The observed retention time deviations (< 0.05 min) and observed mass deviations (< 1 ppm) confirm the excellent instrumental stability for the developed UHPLC-HRMS method. In addition, all procedural blanks were in fact fully blank at the retention time of interest of the target peak, implying that the analytical precautions (see section 3.1.4.) taken were successful.

2.3.2.1.2 Linearity

Weighted linear regression models (Table B10) indicated good linearity (R^2 > 0.99) and no lack of fit (95% confidence interval, F-test, p-value > 0.05) [44].

2.3.2.1.3 Trueness and precision

The recovery ranged for all compounds between 98.5 and 105.8%, with RSDs below 10% (n = 70, independent extractions at 3 different days). These recoveries outperform these reported in related literature, ranging in aquatic matrices from 91.8 to 118% [94]. The precision, encompassing the repeatability and within-laboratory reproducibility complied to the Horwitz equation. The RSDs of repeatability and within-laboratory reproducibility ranged for all the

target compounds, respectively, from 1.6 to 9.5% and 2.4 to 9.9% (Table 5 and Table B9). Comparing our results to reported literature, recovery and within-laboratory reproducibility ranges respectively from 4.1 to 17 % and 4.7 to 12 %, our precision can be considered as good [85].

2.3.2.2 Cross validation on freshwater

The scope of the method was extended and versatility was indicated by performing a cross-validation on fresh tap water samples (Table B11 and Table B12). Tap water was used to evaluate whether the presence of e.g. free chlorine affected the method performance because of matrix effects [46]. When comparing the performance characteristics of freshwater and seawater, similar results were obtained for both matrices. These validation results (inclusive cross validation) suggest that the developed analytical method is robust and applicable to a broad spectrum of aquatic matrices, ranging from very salty to fresh aquatic water.

2.3.3 Analytical strategy for the identification of unknown plasticizers

As the analytical targeted platform was developed on a HR-Q-Orbitrap-analyzer, this also enables the detection of untargeted plasticizer degradation products and metabolites. To elucidate the typical fragmentation profiles and identify characteristic fragments of both the phenols (i.e. alkylphenols and Bisphenol A) and PAEs, the commercially available target standards (prepared in ultrapure water) were fragmented at 20 eV, allowing the simultaneous detection of the pseudo-molecular ion and its associated fragments. Lower collision energies (< 20 eV) resulted in little to no fragmentation of pseudo-molecular ions, whereas higher collision energies (> 20 eV) in the absence of the pseudo-molecular ions.

2.3.3.1 Alkylphenols

Although no truly specific fragments were detected for the branched alkyl substituted phenols, intermediate $[M\text{-}H\text{-}CH_3]^-$ and predominant $[M\text{-}H\text{-}CH_4]^-$ fragments were observed. The observed fragments result from the stepwise loss of a methyl radical (resulting in the intermediate fragment) and a hydrogen radical (leading to the predominant fragment) by the

branched alkyl substituted phenols. Similar fragments $[M\text{-}H\text{-}CH_4]^-$ have also been observed for phenols and structurally related compounds when using high resolution mass spectrometry [95,96]. For the chlorinated APs though, the neutral loss of HCl was noticed resulting in the $[M\text{-}H\text{-}HCl]^-$ fragment. Furthermore, bisphenol A was characterized by the presence of a fragment with *m/z* 133.066 Da, assigned as $[M\text{-}H\text{-}C_6H_6O]^-$. This fragment resulted from the cleavage of the phenyl-alkyl bond followed by the α-cleavage of the ether group. The above-mentioned neutral losses and characteristic fragments were in line with previous fragmentation studies [55,97] and were included in the Python code for tentative identification of unknown AP metabolites or degradation products.

2.3.3.2 Phthalates

The typical fragmentation profiles that were obtained for the protonated pseudo-molecular PAEs $[M+H]^+$ are summarized in Figure 5. The left branch depicts the characteristic peaks associated with the fragmentation of $[M+H]^+$. A first step comprised the elimination of the placeholders, i.e. $[R_1\text{-}H]$ and $[R_2\text{-}H]$, leading to a McLafferty rearrangement product with *m/z* 167.033 Da [98]. Elimination of placeholders has previously been proposed for propyl and high molecular esters [99]. The McLafferty rearrangement is followed by the loss of water $[-H_2O]$, resulting in the protonated phthalic anhydride with m/z 149.023 Da. Subsequently, carbonyl [-CO] is eliminated leading to the formation of protonated benzoic acid with *m/z* 121.029, eventually followed by the loss of oxoketene leading to *m/z* 65.039. This carbonyl loss was not observed for all PAEs. Indeed, for some compounds, the direct generation of *m/z* 65.039 occurred. The right branch represents the specific fragments obtained for placeholders R_1 and R_2, which was only observed for high molecular di-phthalates. For this group, one placeholder was eliminated, followed by the loss of water, resulting in the remaining protonated placeholder. All the afore-mentioned ions (see also Figure 5) were considered specific for the PAEs and incorporated in the Python code to enable tentative identification of unknown phthalate metabolites and degradation products. The strength of the proposed

approach lies within the use of the high resolution (70,000 FWHM) of the MS and the simultaneous detection of 4 different fragmentation ions within a specific ratio.

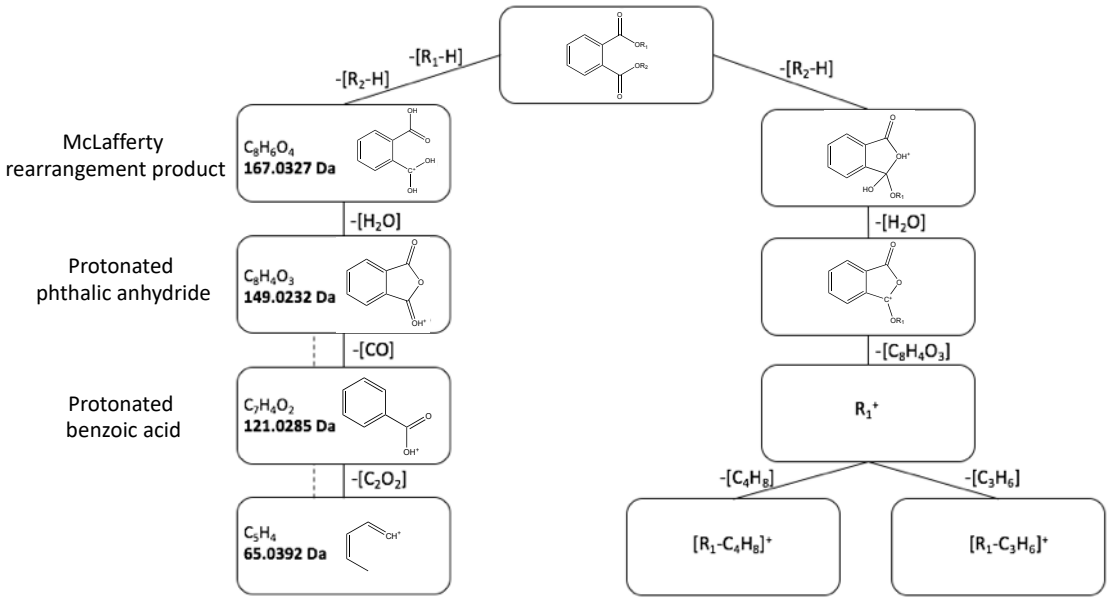


Figure 5. The observed fragmentation patterns for the phthalates in ultrapure water (R1 and R2 represent the placeholders of the plasticizer) at a CE of 20 eV.

2.3.4 Application to seawater samples

To demonstrate the applicability of the developed and validated UHPLC-HRMS method, 24 seawater samples were analyzed in the BPNS, i.e. both targeted quantification and untargeted screening for plasticizers was performed. During targeted analysis, 2 APs and 14 PAEs were detected (Table 6). The highest concentrations were observed for ethylphenol, methylphenol and dibutyl phthalate in the harbor of Oostende (HO). Furthermore, Bisphenol A was not detected at any of the locations, although this was expected due to its extensive use in products and applications and previous reports on its widespread occurrence in human biofluids [100]. In addition to the quantified parent phthalates, also mono-phthalates (i.e. primary phthalate metabolites) were ubiquitously detected at all sampling locations. This may be attributed to the metabolic transformation (and excretion) from aquatic species or human excretion. Since primary phthalate metabolites have been appointed as relevant biomarkers for PAE exposure, both in aquatic organisms [101] and humans [102], our results suggest that phthalate contamination is widely distributed across different trophic levels. Ultimately, it can

be concluded that, the developed and validated HRMS platform, compared to other aquatic screening methodologies [59,103,104], minimized as first the false-positive rate caused by in-house phthalate contamination both for targeted quantification and unknown screening.

For unknown screening purposes, data of the full-scan analysis at a resolution of 70,000 FWHM were subjected to the extraction of relevant unknown components (Compound Discoverer 2.1), which resulted in the detection of 1042 unique unknown components for both polarity modes combined. To elucidate the chemical identity of these unknowns, each extracted component was fragmented by using the PRM scan mode. The generated fragments were screened - using our newly written Python code - on their agreement with characteristic fragments and neutral losses obtained from the commercially available alkylphenols and PAEs (Table B13). In total, 5 % (n = 46) of the unknowns - at the confidence level of Tier 3 according to the Chemical Analysis Working Group & Metabolomics Standards Initiative [82] - could be tentatively identified as plasticizer, i.e. 7 as phenol and 20 as PAE. The following characteristic fragments could be assigned for the phenols: 5 times $[M\text{-}H\text{-}HCl]^-$, 3 times $[M\text{-}H\text{-}CH_3]^-$ and 13 times $[M\text{-}H\text{-}CH_4]^-$. For the PAEs, the following specific fragments were detected for almost every unknown assigned a PAE structure: *m/z* 167.033, 149.023, 121.029 and 65.039. The MS/MS spectra can be consulted in Figure A.13. Finally, during untargeted screening of seawater samples, all target analytes were detected based on the aforementioned ions, confirming that fragmentation occurred similarly in a saline aqueous matrix.

Table 6. Detailed quantified concentrations with the associated standard deviations in ng L^{-1} of the grab samples taken at 4 different locations in the BPNS (51°21'37.78"N; 3° 6'49.01"O (MOW1), 51°20'25.68"N; 3°12'12.11"O (HZ), 51°14'48.59"N; 2°55'39.61"O (Akust39) and 51°13'34.68"N; 2°56'8.00"O (HO)) and 2 different time points (for each time point and each location investigated in threefold, n=3). Only compounds with concentrations above the MQL, for at least one of the sampling locations or time points, were incorporated in this table. Blank cells refer to concentrations below the method detection limits.

Grab samples	Sampling Winter 2016				Sampling Spring 2017			
	MOW1	HZ	Akust39	HO	MOW1	HZ	Akust39	HO
Methylphenol			63 ± 47	215 ± 48		2302 ± 509		6502 ± 1791
ethylphenol	328 ± 150	1518 ± 113	43 ± 3	2508 ± 243	407 ± 440	112 ± 23	593 ± 345	469 ± 159
diethyl phthalate	159 ± 3	336 ± 371		235 ± 111	27 ± 3	43 ± 64	56 ± 62	753 ± 95
dibutyl phthalate		308 ± 297	496 ± 83	2645 ± 250	77 ± 11	791 ± 242	205 ± 35	1502 ± 401
diamyl phthalate					< MQL	< MQL	< MQL	< MQL
benzyl butyl phthalate					79 ± 70	105 ± 29	60 ± 91	343 ± 283
dicyclohexyl phthalate	67 ± 93							
dihexyl phthalate		37 ± 9				23 ± 2		
dibenzyl phthalate		< MQL	< MQL					
diethylhexyl phthalate	269 ± 151	80 ± 54	66 ± 41	100 ± 71	298 ± 145	524 ± 156	218 ± 55	766 ± 314
diisodecyl phthalate		< MQL			< MQL		< MQL	108 ± 49
monomethyl phthalate	235 ± 161				2542 ± 226	158 ± 2	1604 ± 127	< MDL
monobutyl phthalate	176 ± 23	73 ± 28	53 ± 30	165 ± 28	26 ± 10	109 ± 14	292 ± 37	192 ± 8
mono-n-pentyl phthalate	< MQL		< MQL		58 ± 170	25 ± 42		138 ± 100
monobenzyl phthalate								58 ± 1
monoethylhexyl phthalate		399 ± 98			740 ± 391	674 ± 115	423 ± 53	656 ± 123

2.4 CONCLUSIONS

A novel analytical SPE-UHPLC-HR-Q-Orbitrap™-MS method was developed and successfully validated for the simultaneous detection and quantification of 27 known plasticizers and plastics additives in sea and freshwater. Validation demonstrated excellent performance, i.e., stable recoveries ranging from 98.5 to 105.8 %, satisfactory repeatability (RSD < 8%, n = 54) and reproducibility (RSD < 10%, n = 36). The empirical MQL in aquatic

matrices for the phenols, di-phthalates and mono-phthalates ranged respectively from 25 to 200 ng L-1, 10 to 50 ng L^{-1} and 10 to 50 ng L^{-1}. These low MQLs for a broad range of physico-chemical diverse target compounds (log P ranging from 1.1 to 9.9) are vital for the environmental application of this novel method. Indeed, the presented analytical method is the first fulfilling the current need, i.e. the simultaneous quantification of APs, Bisphenol A, PAEs and their primary metabolites at environmental relevant concentrations. The analytical platform also enables simultaneous holistic monitoring of unknown plasticizers by making use of accurate mass data on known characteristic AP fragments and newly discovered PAE fragmentation patterns. Comparing our innovative rapid HRMS platform to other aquatic screening methodologies, the developed platform minimized as a first in its kind the false-positive rate caused by in-house phthalate contamination both for targeted quantification and unknown screening.

In conclusion, our newly developed analytical platform, facilitating the monitoring of a broad range of known and unknown plasticizers and plastics additives, may contribute to national and international legislation, such as the European Water Framework Directive, resulting in better regulations on environmental quality standard levels. Even more, holistic environmental fingerprinting may also contribute to fundamental insights in ocean health, and potential threats on aquatic organisms and humans.

REFERENCES

[1] J.Q. Jiang, Z. Zhou, V.K. Sharma, Occurrence, transportation, monitoring and treatment of emerging micro-pollutants in waste water - A review from global views, Microchem. J. 110 (2013) 292–300. doi:10.1016/j.microc.2013.04.014.

[2] J.P. Laurenson, R. a Bloom, S. Page, N. Sadrieh, Ethinyl estradiol and other human pharmaceutical estrogens in the aquatic environment: a review of recent risk assessment data., AAPS J. 16 (2014) 299–310. doi:10.1208/s12248-014-9561-3.

[3] P.B. Hamilton, E. Nicol, E.S.R. De-Bastos, R.J. Williams, J.P. Sumpter, S. Jobling, et al., Populations of a cyprinid fish are self-sustaining despite widespread feminization of males, BMC Biol. 12 (2014) 1. doi:10.1186/1741-7007-12-1.

[4] T.J. Runnalls, N. Beresford, E. Losty, A.P. Scott, J.P. Sumpter, Several Synthetic Progestins with Diff erent Potencies Adversely Af ect Reproduction of Fish, (2013).

[5] A. Luzio, S.M. Monteiro, E. Rocha, A.A. Fontaínhas-Fernandes, A.M. Coimbra, Development and recovery of histopathological alterations in the gonads of zebrafish (Danio rerio) after single and combined exposure to endocrine disruptors (17α-ethinylestradiol and fadrozole), Aquat. Toxicol. 175 (2016) 90–105. doi:10.1016/j.aquatox.2016.03.014.

[6] J.E. Morthorst, H. Holbech, P. Bjerregaard, Trenbolone causes irreversible masculinization of zebrafish at environmentally relevant concentrations, Aquat. Toxicol. 98 (2010) 336–343. doi:10.1016/j.aquatox.2010.03.008.

[7] CD 2013/39/EU, Directive 2013/39/EU of the European Parliament and of the Council of 12 August 2013, 2013.

[8] K. Fent, Progestins as endocrine disrupters in aquatic ecosystems: Concentrations, effects and risk assessment, Environ. Int. 84 (2015) 115–130. doi:10.1016/j.envint.2015.06.012.

[9] S. Liu, H. Chen, X.-R. Xu, S.-S. Liu, K.-F. Sun, J.-L. Zhao, et al., Steroids in marine aquaculture farms surrounding Hailing Island, South China: Occurrence, bioconcentration, and human dietary exposure, Sci. Total Environ. 502 (2015) 400–407. doi:10.1016/j.scitotenv.2014.09.039.

[10] D.J. Caldwell, F. Mastrocco, T.H. Hutchinson, R. Länge, D. Heijerick, C. Janssen, et al., Derivation of an aquatic predicted no-effect concentration for the synthetic hormone, 17α-ethinyl estradiol, Environ. Sci. Technol. 42 (2008) 7046–7054. doi:10.1021/es800633q.

[11] V. Christen, S. Hickmann, B. Rechenberg, K. Fent, Highly active human pharmaceuticals in aquatic systems: A concept for their identification based on their mode of action, Aquat. Toxicol. 96 (2010) 167–181. doi:10.1016/j.aquatox.2009.11.021.

[12] H. Chang, Y. Wan, S. Wu, Z. Fan, J. Hu, Occurrence of androgens and progestogens in wastewater treatment plants and receiving river waters: Comparison to estrogens, Water Res. 45 (2011) 732–740. doi:10.1016/j.watres.2010.08.046.

[13] J. Jones, W. Mosher, D. K., Current Contraceptive Use in the United States, 2006–2010, and Changes in Patterns of Use Since 1995, 2012. http://europepmc.org/abstract/med/20939159.

[14] S. Kugathas, J.P. Sumpter, Synthetic glucocorticoids in the environment: First results on their potential impacts on fish, Environ. Sci. Technol. 45 (2011) 2377–2383. doi:10.1021/es104105e.

[15] L.-P. Zhang, X.-H. Wang, M.-L. Ya, Y.-L. Wu, Y.-Y. Li, Z. Zhang, Levels of endocrine disrupting compounds in South China Sea., Mar. Pollut. Bull. 85 (2014) 628–33. doi:10.1016/j.marpolbul.2013.12.040.

[16] J.M. Ronan, B. McHugh, A sensitive liquid chromatography/tandem mass spectrometry method for the determination of natural and synthetic steroid estrogens in seawater and marine biota, with a focus on proposed Water Framework Directive Environmental Quality Standards, Rapid Commun. Mass Spectrom. 27 (2013) 738–746. doi:10.1002/rcm.6505.

[17] N.H. Torres, M.M. Aguiar, L.F.R. Ferreira, J.H.P. Américo, Â.M. Machado, E.B. Cavalcanti, et al., Detection of hormones in surface and drinking water in Brazil by LC-ESI-MS/MS and ecotoxicological assessment with Daphnia magna, Environ. Monit. Assess. 187 (2015) 379. doi:10.1007/s10661-015-4626-z.
[18] B. Petrie, J. Youdan, R. Barden, B. Kasprzyk-Hordern, Multi-residue analysis of 90 emerging contaminants in liquid and solid environmental matrices by ultra-high-performance liquid chromatography tandem mass spectrometry, J. Chromatogr. A. 1431 (2016) 64–78. doi:10.1016/j.chroma.2015.12.036.
[19] P.B. Fayad, M. Prévost, S. Sauvé, On-line solid-phase extraction coupled to liquid chromatography tandem mass spectrometry optimized for the analysis of steroid hormones in urban wastewaters, Talanta. 115 (2013) 349–360. doi:10.1016/j.talanta.2013.05.038.
[20] S.X.L. Goh, A. Duarah, L. Zhang, S.A. Snyder, H.K. Lee, Online solid phase extraction with liquid chromatography–tandem mass spectrometry for determination of estrogens and glucocorticoids in water, J. Chromatogr. A. 1465 (2016) 9–19. doi:10.1016/j.chroma.2016.08.040.
[21] T. Anumol, S. a Snyder, Rapid analysis of trace organic compounds in water by automated online solid-phase extraction coupled to liquid chromatography–tandem mass spectrometry, Talanta. 132 (2015) 77–86. doi:10.1016/j.talanta.2014.08.011.
[22] J.O. Tijani, O.O. Fatoba, L.F. Petrik, A review of pharmaceuticals and endocrine-disrupting compounds: Sources, effects, removal, and detections, Water. Air. Soil Pollut. 224 (2013). doi:10.1007/s11270-013-1770-3.
[23] J.O. Ojoghoro, A.J. Chaudhary, P. Campo, J.P. Sumpter, M.D. Scrimshaw, Progesterone potentially degrades to potent androgens in surface waters, Sci. Total Environ. 579 (2016) 1876–1884. doi:10.1016/j.scitotenv.2016.11.176.
[24] B.J. Robinson, J.P.M. Hui, E.C. Soo, J. Hellou, Estrogenic compounds in seawater and sediment from Halifax Harbour, Nova Scotia, Canada., Environ. Toxicol. Chem. 28 (2009) 18–25. doi:10.1897/08-203.1.
[25] The European Parliament and the Council of the European Union, Commission Decision 2002/657/EC implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results, 96/23/Ec Comm. Decis. (2002) 29.
[26] T.E.P. and the C. of the E. Union, Commission directive 2009/90/EC of 31 July 2009 laying down, pursuant to Directive 2000/60/EC of the European Parliament and of the Council, technical specifications for chemical analysis and monitoring of water status, Off. J. Eur. Union. 8 (2009) 36–38.
[27] O. Magnusson, Eurachem Guide: The Fitness for Purpose of Analytical Methods – A Laboratory Guide to Method Validation and Related Topics, 2014. doi:978-91-87461-59-0.
[28] A. Kruve, R. Rebane, K. Kipper, M.-L. Oldekop, H. Evard, K. Herodes, et al., Tutorial review on validation of liquid chromatography–mass spectrometry methods: Part II, Anal. Chim. Acta. 870 (2015) 8–28. doi:10.1016/j.aca.2015.02.016.
[29] A. Kruve, R. Rebane, K. Kipper, M.-L. Oldekop, H. Evard, K. Herodes, et al., Tutorial review on validation of liquid chromatography–mass spectrometry methods: Part I, Anal. Chim. Acta. 870 (2015) 29–44. doi:10.1016/j.aca.2015.02.017.
[30] T. Anumol, S.A. Snyder, Rapid analysis of trace organic compounds in water by automated online solid-phase extraction coupled to liquid chromatography–tandem mass spectrometry, Talanta. 132 (2015) 77–86. doi:10.1016/j.talanta.2014.08.011.
[31] A. International, ASTM D-1141-98(2013) Seawater, 2013.
[32] K. Folens, S. Huysman, S. Van Hulle, G. Du, Chemical and economic optimization of the coagulation-flocculation process for silver removal and recovery from industrial wastewater, Sep. Purif. Technol. 179 (2017) 145–151. doi:10.1016/j.seppur.2017.02.013.
[33] M. Cargouët, D. Perdiz, A. Mouatassim-Souali, S. Tamisier-Karolak, Y. Levi, Assessment of river contamination by estrogenic compounds in Paris area (France),

Sci. Total Environ. 324 (2004) 55–66. doi:10.1016/j.scitotenv.2003.10.035.
[34] M. Borecka, A. Białk-Bielińska, G. Siedlewicz, K. Kornowska, J. Kumirska, P. Stepnowski, et al., A new approach for the estimation of expanded uncertainty of results of an analytical method developed for determining antibiotics in seawater using solid-phase extraction disks and liquid chromatography coupled with tandem mass spectrometry technique, J. Chromatogr. A. 1304 (2013) 138–146. doi:10.1016/j.chroma.2013.07.018.
[35] M. Borecka, G. Siedlewicz, Ł.P. Haliński, K. Sikora, K. Pazdro, P. Stepnowski, et al., Contamination of the southern Baltic Sea waters by the residues of selected pharmaceuticals: Method development and field studies, Mar. Pollut. Bull. 94 (2015) 62–71. doi:10.1016/j.marpolbul.2015.03.008.
[36] E.M. Ferguson, M. Allinson, G. Allinson, S.E. Swearer, K.L. Hassell, Fluctuations in natural and synthetic estrogen concentrations in a tidal estuary in south-eastern Australia, Water Res. 47 (2013) 1604–1615. doi:10.1016/j.watres.2012.12.020.
[37] K. Wille, H.F. De Brabander, E. De Wulf, P. Van Caeter, C.R. Janssen, L. Vanhaecke, Coupled chromatographic and mass-spectrometric techniques for the analysis of emerging pollutants in the aquatic environment, Trends Anal. Chem. 35 (2012) 87–108. doi:10.1016/j.trac.2011.12.003.
[38] C.W. Walker, J.E. Watson, Adsorption of Estrogens on Laboratory Materials and Filters during Sample Preparation, J. Environ. Qual. 39 (2010) 744. doi:10.2134/jeq2009.0017.
[39] D. Guillarme, J. Schappler, S. Rudaz, J.-L. Veuthey, Coupling ultra-high-pressure liquid chromatography with mass spectrometry, TrAC Trends Anal. Chem. 29 (2010) 15–27. doi:10.1016/j.trac.2009.09.008.
[40] J.J. Weisser, C.H. Hansen, R. Poulsen, L.W. Larsen, C. Cornett, B. Styrishave, Two simple cleanup methods combined with LC-MS/MS for quantification of steroid hormones in in vivo and in vitro assays, Anal. Bioanal. Chem. 408 (2016) 4883–4895. doi:10.1007/s00216-016-9575-z.
[41] E.I. Hamelin, W. Bragg, R.L. Shaner, L.L. Swaim, R.C. Johnson, Comparison of high-resolution and tandem mass spectrometry for the analysis of nerve agent metabolites in urine, Rapid Commun. Mass Spectrom. 27 (2013) 1697–1704. doi:10.1002/rcm.6621.
[42] W. Jia, X. Chu, Y. Ling, J. Huang, J. Chang, Analysis of phthalates in milk and milk products by liquid chromatography coupled to quadrupole Orbitrap high-resolution mass spectrometry, J. Chromatogr. A. 1362 (2014) 110–118. doi:10.1016/j.chroma.2014.08.030.
[43] L. Vergeynst, K. K'oreje, P. De Wispelaere, L. Harinck, H. Van Langenhove, K. Demeestere, Statistical procedures for the determination of linearity, detection limits and measurement uncertainty: A deeper look into SPE-LC-Orbitrap mass spectrometry of pharmaceuticals in wastewater, J. Hazard. Mater. 323 (2017) 2–10. doi:10.1016/j.jhazmat.2016.05.077.
[44] E. Commission, Commission Decision 2002/657/EC implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results, 2002. http://www.ecolex.org/ecolex/ledge/view/RecordDetails?id=LEX-FAOC049615&index=documents%5Cnfile://g/R&D/PAPE/articles/Mass Spec/European directive MS IP.pdf.
[45] L.-P. Zhang, X.-H. Wang, M.-L. Ya, Y.-L. Wu, Y.-Y. Li, Z. Zhang, Levels of endocrine disrupting compounds in South China Sea, Mar. Pollut. Bull. 85 (2014) 628–633. doi:10.1016/j.marpolbul.2013.12.040.
[46] M.-Q. Cai, R. Wang, L. Feng, L.-Q. Zhang, Determination of selected pharmaceuticals in tap water and drinking water treatment plant by high-performance liquid chromatography-triple quadrupole mass spectrometer in Beijing, China, Environ. Sci. Pollut. Res. 22 (2015) 1854–1867. doi:10.1007/s11356-014-3473-8.
[47] J.R. Jambeck, Plastic waste inputs from land into the ocean, Clim. Chang. 2014 Impacts, Adapt. Vulnerability. 347 (2015) 1655–1732.

doi:10.1017/CBO9781107415386.010.
[48] Erik, Sebille, Environmental Research Letters A global inventory of small floating plastic debris, Environ. Res. Lett. 10 (2015). http://iopscience.iop.org/article/10.1088/1748-9326/10/12/124006/pdf (accessed November 23, 2017).
[49] P. Lind, B. Zethelius, L. Lind, Circulating Levels of Phthalate Metabolites Are Associated With Prevalent Diabetes in the Elderly, Diabetes Care. 35 (2012) 1519–1524. doi:10.2337/dc11-2396.
[50] E. Fasano, F. Bono-Blay, T. Cirillo, P. Montuori, S. Lacorte, Migration of phthalates, alkylphenols, bisphenol A and di(2-ethylhexyl)adipate from food packaging, Food Control. 27 (2012) 132–138. doi:10.1016/j.foodcont.2012.03.005.
[51] D. Amiridou, D. Voutsa, Alkylphenols and phthalates in bottled waters, J. Hazard. Mater. 185 (2011) 281–286. doi:10.1016/j.jhazmat.2010.09.031.
[52] L. Vidal-Liñán, J. Bellas, N. Salgueiro-González, S. Muniategui, R. Beiras, Bioaccumulation of 4-nonylphenol and effects on biomarkers, acetylcholinesterase, glutathione-S-transferase and glutathione peroxidase, in Mytilus galloprovincialis mussel gilla, Environ. Pollut. 200 (2015) 133–139. doi:10.1016/j.envpol.2015.02.012.
[53] L.N. Vandenberg, S. Ehrlich, S.M. Belcher, N. Ben-Jonathan, D.C. Dolinoy, E.R. Hugo, et al., Low dose effects of bisphenol A, Endocr. Disruptors. 1 (2013) e26490. doi:10.4161/endo.26490.
[54] W. Jia, X. Chu, Y. Ling, J. Huang, J. Chang, Analysis of phthalates in milk and milk products by liquid chromatography coupled to quadrupole Orbitrap high-resolution mass spectrometry, J. Chromatogr. A. 1362 (2014) 110–118. doi:10.1016/j.chroma.2014.08.030.
[55] H. Gallart-Ayala, O. Núñez, P. Lucci, Recent advances in LC-MS analysis of food-packaging contaminants, TrAC - Trends Anal. Chem. 42 (2013) 186–204. doi:10.1016/j.trac.2012.09.017.
[56] A.K. Sakhi, A. Sabaredzovic, E. Cequier, C. Thomsen, Phthalate metabolites in Norwegian mothers and children: Levels, diurnal variation and use of personal care products, Sci. Total Environ. 599–600 (2017) 1984–1992. doi:10.1016/j.scitotenv.2017.05.109.
[57] K.M. Gani, A.A. Kazmi, Phthalate contamination in aquatic environment: A critical review of the process factors that influence their removal in conventional and advanced wastewater treatment, Crit. Rev. Environ. Sci. Technol. 46 (2016) 1402–1439. doi:10.1080/10643389.2016.1245552.
[58] N.I. Rousis, R. Bade, L. Bijlsma, E. Zuccato, J. V. Sancho, F. Hernandez, et al., Monitoring a large number of pesticides and transformation products in water samples from Spain and Italy, Environ. Res. 156 (2017) 31–38. doi:10.1016/j.envres.2017.03.013.
[59] H.P. Singer, A.E. Wössner, C.S. McArdell, K. Fenner, Rapid Screening for Exposure to “non-Target” Pharmaceuticals from Wastewater Effluents by Combining HRMS-Based Suspect Screening and Exposure Modeling, Environ. Sci. Technol. 50 (2016) 6698–6707. doi:10.1021/acs.est.5b03332.
[60] I. Pugajeva, J. Rusko, I. Perkons, E. Lundanes, V. Bartkevics, Determination of pharmaceutical residues in wastewater using high performance liquid chromatography coupled to quadrupole-Orbitrap mass spectrometry, J. Pharm. Biomed. Anal. 133 (2017) 64–74. doi:10.1016/j.jpba.2016.11.008.
[61] A.L. Batt, E.T. Furlong, H.E. Mash, S.T. Glassmeyer, D.W. Kolpin, The importance of quality control in validating concentrations of contaminants of emerging concern in source and treated drinking water samples, Sci. Total Environ. 579 (2017) 1618–1628. doi:10.1016/j.scitotenv.2016.02.127.
[62] L. Canesi, E. Fabbri, Environmental effects of BPA: Focus on aquatic species, Dose-Response. 13 (2015) 155932581559830. doi:10.1177/1559325815598304.
[63] C. Guerranti, E. Grazioli, S. Focardi, M. Renzi, G. Perra, Levels of chemicals in two fish species from four Italian fishing areas, Mar. Pollut. Bull. 111 (2016) 449–452. doi:10.1016/j.marpolbul.2016.07.002.

[64] J. Mathieu-Denoncourt, S.J. Wallace, S.R. de Solla, V.S. Langlois, Influence of Lipophilicity on the Toxicity of Bisphenol A and Phthalates to Aquatic Organisms, Bull. Environ. Contam. Toxicol. 97 (2016) 4–10. doi:10.1007/s00128-016-1812-9.

[65] S. Santangeli, F. Maradonna, M. Zanardini, V. Notarstefano, G. Gioacchini, I. Forner-Piquer, et al., Effects of diisononyl phthalate on Danio rerio reproduction, Environ. Pollut. 231 (2017) 1051–1062. doi:10.1016/j.envpol.2017.08.060.

[66] S. Huysman, L. Van Meulebroek, F. Vanryckeghem, H. Van Langenhove, K. Demeestere, L. Vanhaecke, Development and validation of an ultra-high performance liquid chromatographic high resolution Q-Orbitrap mass spectrometric method for the simultaneous determination of steroidal endocrine disrupting compounds in aquatic matrices, Anal. Chim. Acta. 984 (2017). doi:10.1016/j.aca.2017.07.001.

[67] Y. Liu, Z. Chen, J. Shen, Occurrence and removal characteristics of phthalate esters from typical water sources in northeast China, J. Anal. Methods Chem. 2013 (2013) 419349. doi:10.1155/2013/419349.

[68] D. Gao, Z. Li, Z. Wen, N. Ren, Occurrence and fate of phthalate esters in full-scale domestic wastewater treatment plants and their impact on receiving waters along the Songhua River in China, Chemosphere. 95 (2014) 24–32. doi:10.1016/j.chemosphere.2013.08.009.

[69] J.D. Blair, M.G. Ikonomou, B.C. Kelly, B. Surridge, F.A.P.C. Gobas, Ultra-trace determination of phthalate ester metabolites in seawater, sediments, and biota from an urbanized marine inlet by LC/ESI-MS/MS, Environ. Sci. Technol. 43 (2009) 6262–6268. doi:10.1021/es9013135.

[70] I. González-Mariño, R. Rodil, I. Barrio, R. Cela, J.B. Quintana, Wastewater-Based Epidemiology as a New Tool for Estimating Population Exposure to Phthalate Plasticizers, Environ. Sci. Technol. 51 (2017) 3902–3910. doi:10.1021/acs.est.6b05612.

[71] B.S. Halpern, S. Walbridge, K.A. Selkoe, C. V Kappel, F. Micheli, C. D'Agrosa, et al., A global map of human impact on marine ecosystems., Science. 319 (2008) 948–52. doi:10.1126/science.1149345.

[72] A.D. Tappin, G.E. Millward, The English Channel: Contamination status of its transitional and coastal waters, Mar. Pollut. Bull. 95 (2015) 529–550. doi:10.1016/j.marpolbul.2014.12.012.

[73] J.I.S. Avila, T. Kretzschmar, Simultaneous Determination of Polycyclic Aromatic Hydrocarbons, Alkylphenols, Phthalate Esters and Polychlorinated Biphenyls in Environmental Waters Based on Headspace-Solid Phase Microextraction Followed by Gas Chromatography - Tandem Mass Spectrometry, J. Environ. Anal. Chem. 04 (2017). doi:10.4172/2380-2391.1000226.

[74] A. Paluselli, V. Fauvelle, N. Schmidt, F. Galgani, S. Net, R. Sempéré, Distribution of phthalates in Marseille Bay (NW Mediterranean Sea), Sci. Total Environ. 621 (2018) 578–587. doi:10.1016/j.scitotenv.2017.11.306.

[75] J. Sánchez-Avila, J. Bonet, G. Velasco, S. Lacorte, Determination and occurrence of phthalates, alkylphenols, bisphenol A, PBDEs, PCBs and PAHs in an industrial sewage grid discharging to a Municipal Wastewater Treatment Plant, Sci. Total Environ. 407 (2009) 4157–4167. doi:10.1016/j.scitotenv.2009.03.016.

[76] A.L. Heffernan, K. Thompson, G. Eaglesham, S. Vijayasarathy, J.F. Mueller, P.D. Sly, et al., Rapid, automated online SPE-LC-QTRAP-MS/MS method for the simultaneous analysis of 14 phthalate metabolites and 5 bisphenol analogues in human urine, Talanta. 151 (2016) 224–233. doi:10.1016/j.talanta.2016.01.037.

[77] M. Del Carlo, A. Pepe, G. Sacchetti, D. Compagnone, D. Mastrocola, A. Cichelli, Determination of phthalate esters in wine using solid-phase extraction and gas chromatography–mass spectrometry, Food Chem. 111 (2008) 771–777. doi:10.1016/J.FOODCHEM.2008.04.065.

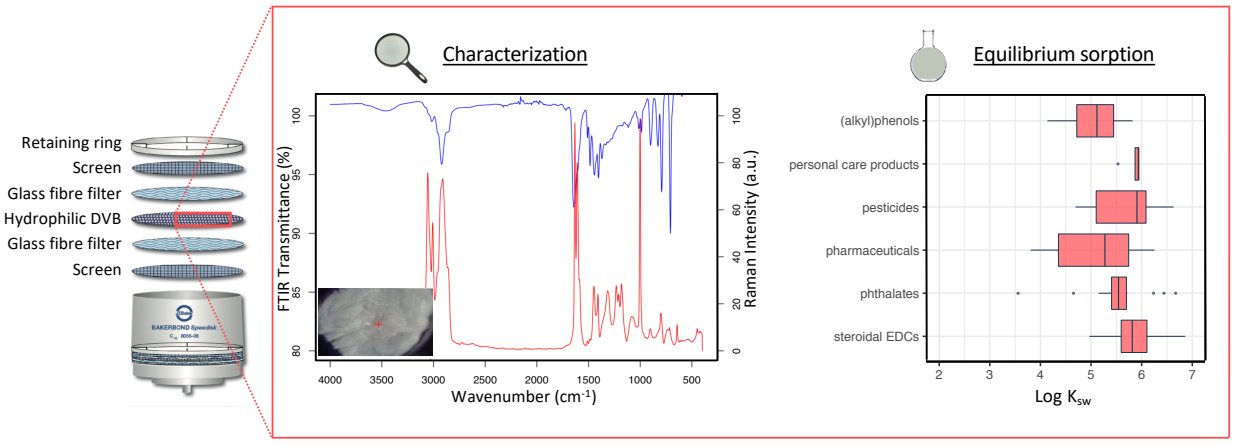
[78] T.F.T. Omar, A. Ahmad, A.Z. Aris, F.M. Yusoff, Endocrine disrupting compounds (EDCs) in environmental matrices: Review of analytical strategies for pharmaceuticals, estrogenic hormones, and alkylphenol compounds, TrAC - Trends Anal. Chem. 85

(2016) 241–259. doi:10.1016/j.trac.2016.08.004.
[79] J. Sánchez-avila, J. Bonet, G. Velasco, S. Lacorte, Science of the Total Environment Determination and occurrence of phthalates , alkylphenols , bisphenol A , PBDEs , PCBs and PAHs in an industrial sewage grid discharging to a Municipal Wastewater Treatment Plant, Sci. Total Environ. 407 (2009) 4157–4167. doi:10.1016/j.scitotenv.2009.03.016.
[80] D.A. Markham, J.M. Waechter, M. Wimber, N. Rao, P. Connolly, J.C. Chuang, et al., Development of a method for the determination of bisphenol a at trace concentrations in human blood and urine and elucidation of factors influencing method accuracy and sensitivity, J. Anal. Toxicol. 34 (2010) 293–303. doi:10.1093/jat/34.6.293.
[81] The European Parliament and the Council of the European Union, Directive 2008/105/EC of 16 December 2008 on environmental quality standards in the field of water policy, amending and subsequently repealing Council Directives 82/176/EEC, 83/513/EEC, 84/156/EEC, 84/491/ECC, 86/280/ECC and amending Directive 2000/60/EC, Off. J. Eur. Union. L348 (2008) 84–97. doi:http://eur-lex.europa.eu/legal-content/EN/TXT/?uri=celex:32008L0105.
[82] E.L. Schymanski, J. Jeon, R. Gulde, K. Fenner, M. Ruff, H.P. Singer, et al., Identifying small molecules via high resolution mass spectrometry: Communicating confidence, Environ. Sci. Technol. 48 (2014) 2097–2098. doi:10.1021/es5002105.
[83] T. Wu, C. Wang, X. Wang, H. Xiao, Q. Ma, Q. Zhang, Comparison of UPLC and HPLC for Analysis of 12 Phthalates, Chromatographia. 68 (2008) 803–806. doi:10.1365/s10337-008-0788-y.
[84] M.I. Churchwell, N.C. Twaddle, L.R. Meeker, D.R. Doerge, Improving LC-MS sensitivity through increases in chromatographic performance: Comparisons of UPLC-ES/MS/MS to HPLC-ES/MS/MS, J. Chromatogr. B Anal. Technol. Biomed. Life Sci. 825 (2005) 134–143. doi:10.1016/j.jchromb.2005.05.037.
[85] C. Esteve, L. Herrero, B. Gómara, J.E. Quintanilla-lópez, Talanta Fast and simultaneous determination of endocrine disrupting compounds by ultra-high performance liquid chromatography – tandem mass spectrometry, Talanta. 146 (2016) 326–334. doi:10.1016/j.talanta.2015.08.064.
[86] E. Ates, K. Mittendorf, H. Senyuva, An Automated Online TurboFlow™ Cleanup LC/MS/MS Method for the Determination of 11 Plasticizers in Beverages and Milk, J. AOAC Int. 96 (2013) 1092–1100. doi:10.5740/jaoacint.12-299.
[87] B.O. Keller, J. Sui, A.B. Young, R.M. Whittal, Interferences and contaminants encountered in modern mass spectrometry, Anal. Chim. Acta. 627 (2008) 71–81. doi:10.1016/J.ACA.2008.04.043.
[88] A. Bergé, J. Gasperi, V. Rocher, L. Gras, A. Coursimault, R. Moilleron, Phthalates and alkylphenols in industrial and domestic ef fl uents : Case of Paris conurbation (France), Sci. Total Environ. 488–489 (2014) 26–35. doi:10.1016/j.scitotenv.2014.04.081.
[89] Y. Jeong, A. Schäffer, K. Smith, Equilibrium partitioning of organic compounds to OASIS HLB as a function of compound concentration, pH, temperature and salinity, Chemosphere. 174 (2017) 297–305. doi:10.1016/j.chemosphere.2017.01.116.
[90] M. Yang, S. Fazio, D. Munch, P. Drumm, Impact of methanol and acetonitrile on separations based on π-π interactions with a reversed-phase phenyl column, J. Chromatogr. A. 1097 (2005) 124–129. doi:10.1016/j.chroma.2005.08.028.
[91] A. Guart, F. Bono-Blay, A. Borrell, S. Lacorte, Effect of bottling and storage on the migration of plastic constituents in Spanish bottled waters, Food Chem. 156 (2014) 73–80. doi:10.1016/j.foodchem.2014.01.075.
[92] C.S. Clendinen, G.S. Stupp, R. Ajredini, B. Lee-McMullen, C. Beecher, A.S. Edison, An overview of methods using 13C for improved compound identification in metabolomics and natural products, Front. Plant Sci. 6 (2015) 611. doi:10.3389/fpls.2015.00611.
[93] European Commission, Directive 2008/105/EC of 16 December 2008 on environmental quality standards in the field of water policy, amending and subsequently repealing Council Directives 82/176/EEC, 83/513/EEC, 84/156/EEC, 84/491/ECC, 86/280/ECC and amending Directive 2000/60/EC, Off. J. Eur. Union. L348 (2008) 84–97.

doi:http://eur-lex.europa.eu/legal-content/EN/TXT/?uri=celex:32008L0105.
[94] W. Chen, H. Huang, C.E. Chen, S. Qi, O.R. Price, H. Zhang, et al., Simultaneous determination of 20 trace organic chemicals in waters by solid-phase extraction (SPE) with triple-quadrupole mass spectrometer (QqQ-MS) and hybrid quadrupole Orbitrap high resolution MS (Q-Orbitrap-HRMS), Chemosphere. 163 (2016) 99–107. doi:10.1016/j.chemosphere.2016.07.080.
[95] N. Fabregat-Cabello, J. Pitarch-Motellón, J. V Sancho, M. Ibáñez, A.F. Roig-Navarro, Method development and validation for the determination of selected endocrine disrupting compounds by liquid chromatography mass spectrometry and isotope pattern deconvolution in water samples. Comparison of two extraction techniques, Anal. Methods. 8 (2016) 2895–2903. doi:10.1039/c6ay00221h.
[96] L. Bajpai, M. Varshney, C.N. Seubert, S.M. Stevens, J. V Johnson, R.A. Yost, et al., Mass spectral fragmentation of the intravenous anesthetic propofol and structurally related phenols, J. Am. Soc. Mass Spectrom. 16 (2005) 814–824. doi:10.1016/j.jasms.2005.02.009.
[97] H. Gallart-Ayala, E. Moyano, M.T. Galceran, Liquid chromatography/multi-stage mass spectrometry of bisphenol A and its halogenated derivatives, Rapid Commun. Mass Spectrom. 21 (2007) 4039–4048. doi:10.1002/rcm.3307.
[98] Y.A. Jeilani, B.H. Cardelino, V.M. Ibeanusi, Positive chemical ionization triple-quadrupole mass spectrometry and ab initio computational studies of the multi-pathway fragmentation of phthalates, J. Mass Spectrom. 45 (2010) 678–685. doi:10.1002/jms.1761.
[99] A.G. Harrison, Chemical Ionization Mass Spectrometry, 1983. https://books.google.be/books?hl=nl&lr=&id=HJ-j71b7yfIC&oi=fnd&pg=PA1&dq=a.g.+harrison+chemical+ionization+mass+spectrometry+boca&ots=y5qAeXI9M-&sig=FSjE5Z4geD2rSwGNpchXUOy7K-Q (accessed January 12, 2018).
[100] S.D. Fox, R.T. Falk, T.D. Veenstra, H.J. Issaq, Quantitation of free and total bisphenol A in human urine using liquid chromatography-tandem mass spectrometry, J. Sep. Sci. 34 (2011) 1268–1274. doi:10.1002/jssc.201100087.
[101] X. Hu, Y. Gu, W. Huang, D. Yin, Phthalate monoesters as markers of phthalate contamination in wild marine organisms, Environ. Pollut. 218 (2016) 410–418. doi:10.1016/j.envpol.2016.07.020.
[102] A. Ramesh Kumar, P. Sivaperumal, Analytical methods for the determination of biomarkers of exposure to phthalates in human urine samples, TrAC - Trends Anal. Chem. 75 (2016) 151–161. doi:10.1016/j.trac.2015.06.008.
[103] M. Ruff, M.S. Mueller, M. Loos, H.P. Singer, Quantitative target and systematic non-target analysis of polar organic micro-pollutants along the river Rhine using high-resolution mass-spectrometry - Identification of unknown sources and compounds, Water Res. 87 (2015) 145–154. doi:10.1016/j.watres.2015.09.017.
[104] J. Cotton, F. Leroux, S. Broudin, M. Poirel, B. Corman, C. Junot, et al., Development and validation of a multiresidue method for the analysis of more than 500 pesticides and drugs in water based on on-line and liquid chromatography coupled to high resolution mass spectrometry, Water Res. 104 (2016) 20–27. doi:10.1016/j.watres.2016.07.075.

III

Passive sampling - Hydrophilic divinylbenzene for equilibrium sorption of emerging organic contaminants in aquatic matrices



Adapted from:

(1) Huysman S, Vanryckegem F, De Paepe E, Smedes F, A. Haughey S, T. Elliott C, Demestere K, Vanhaecke L. - submitted to Environmental Science and Technology

ABSTRACT

Hydrophilic divinylbenzene (DVB) (Bakerbond™) has surfaced as a promising sorbent for active sampling of analytes from aqueous matrices over a very broad polarity range. Given this, hydrophilic DVB may likewise offer potential for passive sampling, if sorbent-water partitioning coefficients (K_{sw}) were to be available. In this work, static exposure batch experiments were performed to quantitatively study the equilibrium sorption of 131 environmentally relevant organic contaminants (P values ranging from -1.30 to 9.85) on hydrophilic DVB. The superior affinity of hydrophilic DVB for compounds with a broad polarity range was confirmed by functional FTIR and Raman characterization, demonstrating the presence of carboxyl moieties. Concentration effects were studied by increasing compound concentrations in mixture experiments, and resulted for the steroidal EDCs in higher K_{sw}, while lower K_{sw} were obtained for the (alkyl)phenols, personal care products, pesticides, pharmaceuticals and phthalates. Nevertheless, Ksw remained constant in said design for equilibrium water concentrations at environmentally relevant seawater levels. Analysis of thermodynamic parameters (change in enthalpy, entropy and Gibbs free energy) revealed the nature of the main partitioning processes. While polar (log P < 4) compounds were mainly served by physisorption, non-polar (log P > 4) compounds also exhibited binding by chemisorption. In conclusion, this research facilitates the future application of hydrophilic DVB for active as well as passive sampling in the analysis of organic contaminants for monitoring purposes but also for toxicity testing.

1 INTRODUCTION

The ever-increasing number of emerging contaminants released into our (aquatic) environment stresses the need for the deployment of efficient monitoring strategies [1]. In this context, the use of polymeric sorbents has surfaced as a highly valuable strategy for active and passive sampling of organic compounds from aquatic matrices [2,3]. In active sampling, polymeric sorbents are widely used in solid-phase extraction (SPE) cartridges for the enrichment of contaminants and/or clean-up of aquatic matrices [2]. In recent years, however, the number of publications on the application of polymeric sorbents and materials in passive sampling studies is steadily increasing.

At present, a number of polymeric materials have been applied for the monitoring of emerging contaminants using passive sampling based approaches [3,4]. However, most studies focus on specific polymeric passive samplers efficiently binding compounds within a specific polarity range [5]. In passive sampling, the function of a sampler as an infinite sink is determined by the affinity towards the envisaged analytes, which is governed by the polarity, (inversely related to log P) and chemical functionalities of both. Indeed, silicones (mainly polydimethylsiloxane, PDMS) invoke the uptake of non-polar chemicals (log P > 4) [6,7], while Chemcatchers® SDB-RPS (sulfonated divinylbenzene - reversed phase sulfonate) mainly accumulate more polar chemicals (log P < 4) and Chemcatchers® C_{18} sample compounds ranging from moderately polar to moderately non-polar, i.e. log P ranging between 0.9 and 5.8 [8,9]. In this context, the Oasis HLB® co-polymer has gained in popularity and was recently incorporated in different POCIS configurations [10,11], as it permits the accumulation of very polar to moderately non-polar compounds (log P ranging from -1.6 up to 5) [12]. Its affinity towards hydrophobic compounds (log P > 5) however still remains a hurdle [5]. In this work, we propose a 'novel' sorbent, i.e. hydrophilic DVB, that empowers sampling of non-polar compounds while still sufficiently capturing the very polar compounds. To the best of our knowledge, the potential of hydrophilic DVB for passive sampling remains unexamined as opposed to the frequently applied Oasis HLB® sorbent. Moreover, higher extraction

efficiencies were obtained using hydrophilic DVB for non-polar compounds (log P > 4) during active sampling as compared to Oasis HLB® [13]. Hence, investigating the underlying mechanisms of said hydrophilic DVB sorbent in both active (SPE) and passive sampling seems promising. Indeed, there is no information available on the surface chemistry of hydrophilic DVB neither on the partitioning behaviour of organic contaminants between water and hydrophilic DVB [14]. Furthermore, hydrophilic DVB is commercially available in a robust housing, i.e. Bakerbond Speedisks®, which simplifies its application for passive sampling of the aquatic environment.

Therefore, the goal of this work was to investigate the equilibrium sorption behaviour of a broad range of aqueous organic contaminants on hydrophilic DVB. The specific objectives included (i) revealing the surface chemistry of hydrophilic DVB, (ii) determining the K_{sw} of a mixture of 131 environmentally relevant organic contaminants at field concentrations, (iii) examining sorption linearity of the target organic contaminants by increasing compound concentrations, and (iv) assessing the impact of relevant environmental parameters (i.e. temperature, pH and salinity) on K_{sw}. Ultimately, this research intends to reveal the underlying mechanisms of hydrophilic DVB sorption in active and passive sampling.

2 MATERIALS AND METHODS

2.1 Chemicals and materials

In this study, standards of 131 environmentally relevant organic contaminants (log P ranging from -1.30 to 9.85) were purchased at Bayer (Germany), Fluka (Belgium), Sigma Aldrich (St. Louis, MO, USA), Steraloids Inc (Newport, RI, USA), Lipomed GmbH (Germany), MpBio (Belgium) and TRC (Canada), covering 6 major classes, i.e. 4 alkylphenols, 5 personal care products, 25 pesticides, 32 pharmaceuticals, 15 phthalates and 50 steroidal endocrine disrupting compounds (EDCs). The selected organic contaminants were based on legislative frameworks and directives for protecting surface and marine waters, i.e. the U.S. Clean Water Act, the EU WFD watchlist, OSPAR, Reach and Norman [15–19]. Primary stock solutions and

standard mixtures were prepared in pure methanol (CH_3OH) or in a mixture of methanol/water (10/90, v/v %) with 0.1% (v/v %) formic acid and 0.1 g L^{-1} $Na_2EDTA.2H_2O$, the latter only for pesticides, pharmaceuticals and personal care products, thereby attaining concentrations between 0.01 and 1000 ng μL^{-1}. Solutions were stored in dark glass bottles at -20°C. Organic solvents were of Optima UPLC-MS grade, purchased from Fisher Scientific (Loughborough, UK). The inorganic salts, used to prepare reference seawater, were supplied by Sigma Aldrich (St. Louis, MO, USA) and processed according to ASTM D-1141 [20]. The hydrophilic divinylbenzene (DVB) Speedisks® and Oasis® HLB cartridges (200 and 500 mg) were purchased from Filterservice (Eupen, Belgium) and Waters (Brussels, Belgium), respectively.

2.2 Static exposure batch experiments

A classic static exposure batch system, which has previously been applied for passive sampling studies [21,22], was established under controlled conditions of continuous stirring (100 rpm), temperature (experiment dependent) and absence of light. The latter was applied to determine the K_{sw} of a mixture of the 131 selected contaminants between artificial seawater and the hydrophilic DVB sorbent. It was also studied how compound concentration and environmental parameters affect the K_{sw}-values. A schematic representation of the different batch experiments is depicted in Figure 1. Aqueous suspensions, containing 25 mg hydrophilic DVB L^{-1} were used in all batch experiments. The sorbent/water-ratio was determined based on preliminary experiments. A higher sorbent/water-ratio disabled us to quantify the spiked compounds, as aqueous equilibrium concentrations were below the analytical detection limits. Compound mixtures were spiked in 1L glass beakers closed with aluminium foil [23] to prevent evaporation. At the end of each batch test, the beaker's content, containing both the aqueous and sorbent phases, was filtered over a Whatman GF/D glass fibre filter coupled in series with a packed SPE cartridge to extract the analytes from the aqueous phase (see supplementary information, Figure C1). The glass fibre filter with sorbent phase and packed SPE cartridge were separately eluted and analysed, to provide sorbent and aqueous concentrations,

respectively. The subsequent sample preparation and analysis were optimised for the different compound groups of interest (see Section 2.3.2).

To evaluate potential adsorption to glass walls and/or hydrolysis of the spiked compounds during the batch experiments, reference glass beakers were included in the experimental set-up. These reference glass beakers, containing only the initial nominal concentration of the compounds and no sorbent, were subjected to similar conditions as beakers containing the spiked concentration with sorbents."

2.2.1 Experimental determination of K_{sw}

The K_{sw} were determined at neutral pH conditions and at a temperature of 8°C. The spike concentration for the target compounds was based on environmentally relevant levels but sufficiently high as to enable measurements above the methods' detection limits after sorbent sorption. As such, the initial nominal concentration was set at 1.5 nmol L^{-1} for each compound. The residual concentrations in water and the amounts accumulated by the sorbent were measured at 0, 1, 2, 4, 6, 8, 12, 24, 48, 96 and 168h. For each time point, a separate 1L beaker was used.

2.2.2 Partitioning at different compound concentrations

To investigate the effect of compound concentration on the K_{sw}, at a contact time of 48h, multiple equilibrations were performed under similar conditions at various concentration levels, i.e. 0.75, 1.5, 3.0, 6.0, 12, 24 and 48 nmol L^{-1}, This experiment was executed at a water temperature of 8°C and under neutral pH conditions.

2.2.3 Partitioning under different environmental conditions

To evaluate the impact of varying environmental conditions on the K_{sw} (48h contact time), three parameters were investigated within environmental relevant ranges, i.e. pH, temperature and salinity respectively ranging from 5 to 8, from 4 to 16°C, and from 0 to 35 psu. Response surface modelling (RSM), and more specifically the Box-Behnken experimental design,

enabled to fit quadratic models to the experimental log K_{sw}, which contained main, quadratic and interaction factors related to the selected environmental parameters.

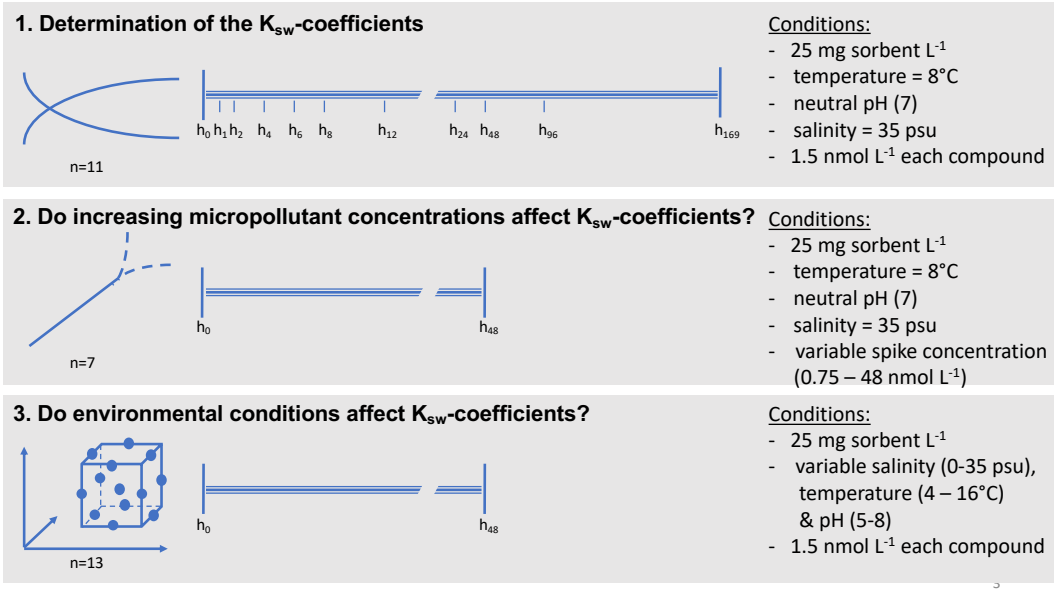


Figure 1. Schematic representation of the different experimental conditions used to determine the Ksw. The large ticks represent the start and end of the experiments, with the corresponding sampling of water and sorbent phase. The small ticks represent intermediate sampling points of water and sorbent phase. The 'h' corresponds to the contact time in hours.

2.3 Analytical methods

2.3.1 Sorbent characterization

Sorbent characterization was performed to reveal the chemical properties of the hydrophilic DVB surface and to compare the latter with the frequently used Oasis HLB®. This was achieved by the use of a Thermo Scientific Nicolet iS50 spectrophotometer (Thermo Fisher Sicentific, Dublin, Ireland) to obtain the FTIR (Fourier transform infrared), Raman and NIR (Near-infrared) spectra within the range of 400 - 4000 cm^{-1}, 400 - 4000 cm^{-1} and 3000 – 12000 cm^{-1}, respectively. Other acquisition parameters were: number of scans: 256, number of background scans: 256, background gain: 4.0, and resolution: 4.0 (FTIR and NIR) / 8.0 (Raman). Acquisition was repeated 3 times, and spectral data were averaged prior to further data processing.

2.3.2 Sample analysis

Organic contaminant quantification in the aqueous and sorbent phases was performed using three in-house developed and validated analytical methods, consisting of the appropriate extraction followed by an optimized UHPLC-HR-Q-Orbitrap™-MS methodology. Chromatographic separation was achieved using reversed phase chromatography with gradient elution using a Hypersil Gold column (1.9 µm, 50/100 x 2.1 mm). Analyte detection was carried out on a Q-Exactive™ benchtop HRMS (Thermo Fisher Scientific, San-Francisco, USA). Details regarding the sample analysis have been published earlier [13,23,24], and only the main differences are briefly described in the supplementary information (section A).

2.4 Data treatment and analysis

Spectroscopic data were extracted from the FTIR, Raman and NIR using TQ Analyst 8.6.12 (Thermo Fisher Scientific, Dublin, Ireland). Targeted processing of full-scan data, obtained by the Q-Exactive HRMS, including the identification and quantification of target compounds, was executed by XCalibur™ 4.0 software (Thermo Fisher Scientific). The software program JMP 12.0 (SAS Institute Inc, Cary, USA) was used to evaluate and model the appropriate RSM design Box-Behnken Design. Graphics were produced by using R (Version 3.4).

2.4.1 Determination of K_{sw}

K_{sw} were determined using the above-described static exposure design and calculated by the respective ratio of the concentration measured in the sorbent ($C_{s,t}$; nmol g_s^{-1}) and water ($C_{w,t}$; nmol L^{-1}) phase at equilibrium (Eq. 1.):

$$K_{sw} = \frac{C_{s,t=equilibrium}}{C_{w,t=equilibrium}} \quad (1)$$

[25].

2.4.2 Adsorption modelling

Adsorption isotherms were investigated by applying the Freundlich model (see Eq. 2) [26], where q_e (nmol g_s^{-1}) is the mass of accumulated compound by the sorbent at equilibrium, which equals $C_{s,t}$ as described under 2.4.1, K_F ((nmol g_s^{-1})/(nmol L^{-1})^n) the Freundlich constant,

C_e (nmol L^{-1}) the compound concentration in the water phase at equilibrium, equivalent to $C_{w,t}$ described under 2.4.2, and n the dimensionless Freundlich exponent.

The thermodynamic parameters [26], i.e. change in Gibbs free energy ($\Delta G_{288,15K}$), enthalpy (ΔH) and entropy (ΔS), were determined using the van't Hoff (see Eq. 3) and change in Gibbs free energy equations (see Eq. 4). R represents the universal gas constant (8.314 J K^{-1} mol^{-1}) and T the absolute temperature (K).

$$q_e = K_F C_e^n \quad (2)$$

$$\ln\left(K^\circ\right) = -\frac{\Delta H^\circ}{RT} + \frac{\Delta S^\circ}{R} \quad (3)$$

$$\Delta G_{288.15K} = \Delta H^\circ - T\Delta S^\circ \quad (4)$$

3 RESULTS AND DISCUSSION

3.1 Sorbent characterization

To the best of our knowledge, the surface chemistry of hydrophilic DVB has not been studied earlier. This work relied on spectral analysis (i.e. FTIR, Raman and NIR) to unravel surface functionalities of hydrophilic DVB. Additionally, the co-polymer poly(divinylbenzene-co-N-vinylpyrrolidone), also more familiar under the tradename Oasis® HLB, was analysed for comparison. FTIR and Raman spectra are presented in Figure 2, whilst NIR spectra can be found in supplementary Figure C2.

Multivariate statistical analysis of the obtained FTIR spectra demonstrated significant differences between the two sorbents (p-value < 0.05, $R^2(X)(cum)$ = 0.989, $R^2(Y)$ = 0.99, $Q^2(cum)$ = 0.974). The main spectral differences consisted of FTIR peaks at 1642 and 1403 cm^{-1} for hydrophilic DVB, and 1684 cm^{-1} for Oasis® HLB. An FTIR peak at 1642 cm^{-1} has been related to the presence of water adsorbed to amorphous regions of a polymer [27,28], suggesting the presence of hydroxyl [-OH] and/or ketone [-C=O] groups. The FTIR peak at 1403 cm^{-1} of hydrophilic DVB represents the presence of carboxyl [-COOH] groups [29,30].

For Oasis® HLB, the FTIR peak at 1684 cm^{-1} corresponds to the amide [C-N] stretching vibration of the pyrrolidine ring present in the PVP (poly-N-vinylpyrrolidone) moiety.

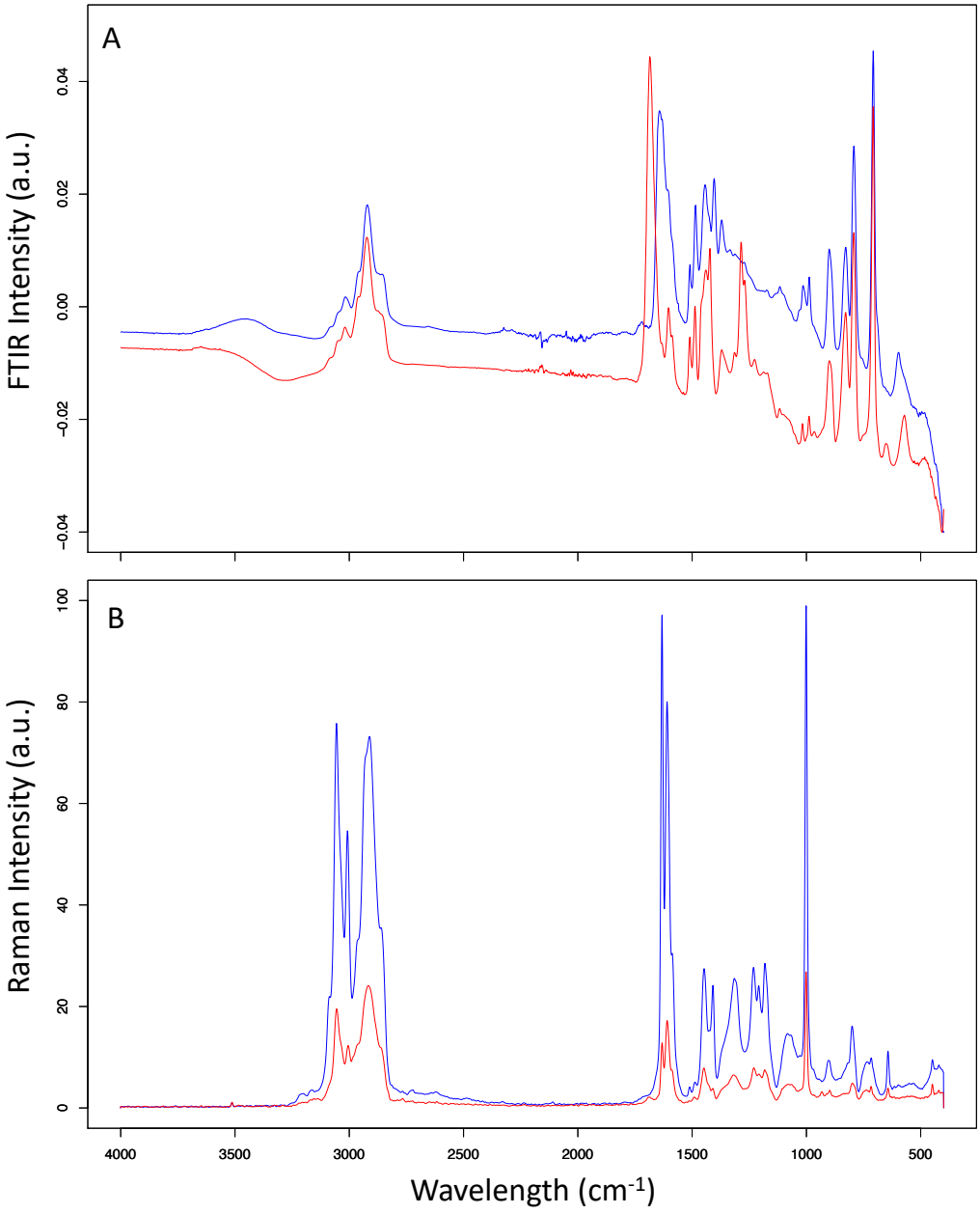


Figure 2. Averaged FTIR (A, n = 3 for each sorbent) and Raman (B, n = 3 for each sorbent) spectroscopic analysis of hydrophilic DVB (Bakerbond™) (Blue) and Oasis® HLB (red).

As no additional peak was observed at 3400 cm^{-1} and because the FTIR amide peak was observed within the 1650 - 1750 cm^{-1} range, the presence of a tertiary amide was concluded. In addition, for both sorbents peaks at 1600 and 1510 cm^{-1} confirmed the occurrence of aromatic hydrocarbons [C=C] belonging to the divinylbenzene co-polymer. Complementary to the FTIR spectra, the Raman spectra also provided qualitative and quantitative information on the functional groups of the two sorbents (Figure 2). Raman analysis revealed and confirmed the presence of carboxyl [-COOH] moieties in the hydrophilic DVB co-polymer, i.e. a peak at 1409 cm^{-1} was observed [31]. In addition, the occurrence of the Oasis HLB® vinyl-groups [CH_2=CH_2] was confirmed as we observed a Raman peak at 1229 cm^{-1}. Aside from the Raman peaks at 1409 cm^{-1} and 1229 cm^{-1}, in general, similar spectra were observed for both the hydrophilic DVB and Oasis® HLB co-polymers. However, more intense signals were marked for the hydrophilic DVB functional groups, revealing a higher degree of cross-linkage and functionalisation for hydrophilic DVB as compared to Oasis® HLB, offering an explanation for the higher extraction efficiencies for steroidal EDCs, pharmaceuticals, pesticides and personal care products that have generally been observed for hydrophilic DVB versus Oasis® HLB [13,24]. No significant (p-value < 0.05) differences were observed between the NIR spectra of hydrophilic DVB and Oasis® HLB.

3.2 Equilibrium partitioning between water and hydrophilic DVB

3.2.1 Mass balances

The applied analytical methods for the quantification of the 131 organic contamiants studied in this work, have been extensively and successfully validated as reported earlier [13,23,24]. To assure that decreasing contaminant concentrations measured in the aqueous phase during the aforementioned batch tests were merely caused by sorption on the sorbent, mass balances were calculated for every contaminant. This was achieved by comparing the initially spiked contaminant quantity to the sum of the fractions remaining in the water and sorbent phases [32]. Average mass balances (%) and corresponding analytical repeatabilities (% RSD) of the different time points in equilibrium (constant K_{sw}) for each studied contaminant

are listed in Table C1. For the (alkyl)phenols, personal care products, pesticides, pharmaceuticals, phthalates and steroidal EDCs, averaged class-specific mass balances equalled 76±4%, 47±4%, 58±5%, 59±5, 79±4% and 75±9%, while averaged class-specific repeatability was calculated to 5%, 9%, 9%, 8%, 5% and 11%, respectively. As repeatabilities were better than 15%, it may be concluded that the batch sorption experiments were consistent and reproducible.

From these data, it can also be deduced that all compounds experienced losses (not related to sorption) of < 20% after 168h, which can be assigned to adsorption to glass walls and/or hydrolysis as was observed by Jeong et al. (2017) [33].

3.2.2 Determination of the K_{sw}

All organic contaminants studied in this work reached sorption equilibrium between 12 and 24h. The calculated log transformed K_{sw} are listed for the individual components in Table C2, and are depicted per class of compounds in Figure 3. K_{sw} ranged over more than 3 orders of magnitude, from log K_{sw} 3.81 for nalidixic acid (pharmaceutical) to 6.93 for terbuthylazine (pesticide).

To the best of the author's knowledge, only one study of Jeong et al. (2017) determined the sorption of 28 organic compounds to naked SPE sorbent (i.e. OasisTM HLB) in a static exposure design. The log Ksw-values of specific compounds obtained in this study, i.e. metoprolol (5.38), isoproturon (6.16), carbamazepine (6.15), flufenacetate (5.92), diuron (6.48), atrazine (6.09), sulfamethoxazole (3.96), simazine (5.91) and terbutryn (6.18) are comparable (except for sulfamethoxazole) or slightly higher (log K_{sw}-difference ranged between 0.13 and 1.22 log unit), than those reported by Jeong et al. (2017) (5.15; 6.03; 5.64; 5.23; 5.26; 5.29; 4.44; 5.35 and 5.39, respectively). These data suggest that the investigated polar compounds (log P < 4) have a slightly higher sorption tendency (higher K_{sw} values, availability of more divinylbenzene groups (see characterization section 3.1)) to hydrophilic DVB as compared to the Oasis^{TM} HLB sorbent. This agrees with the functional characterization data, which demonstrated that hydrophilic DVB comprises hydrophilic moieties with a higher

polarity as does Oasis® HLB (see section 3.1), i.e. carboxyl [-COOH] as opposed to N-vinylpyrrolidone groups. Comparing hydrophilic DVB to other SPE-based sampling devices (with sorbent casing) also corroborates the conclusion that hydrophilic DVB covers a much broader polarity range (log P ranging from -1.30 to 9.85) than any previously tested sorbent. Ahrens et al. (2015) tested a broad spectrum of organic compounds with log P values ranging from -1.7 to 6.9, and this for 5 different polymer-based passive sampling devices, to conclude that the sorption of organic compounds to each device was optimal within a specific polarity range. Silicone rubbers, Chemcatcher SDB-RPS, Chemcatcher C18, POCIS A and POCIS B, effectively sampled organic compounds with log P values ranging respectively from 0.5 to 5.8, -1.3 to 4.0, 0.9 to 5.8, -1.6 to 5.5 and -1.6 to 5.5. Since the above-mentioned study used different types of limiting membranes, K_{sw} cannot be compared to our work. Comparing our results with the study of Ahrens et al. (2015), however confirms that hydrophilic DVB is a more cross-linked and functionalised polymer towards hydrophobic compounds than Oasis™ HLB.

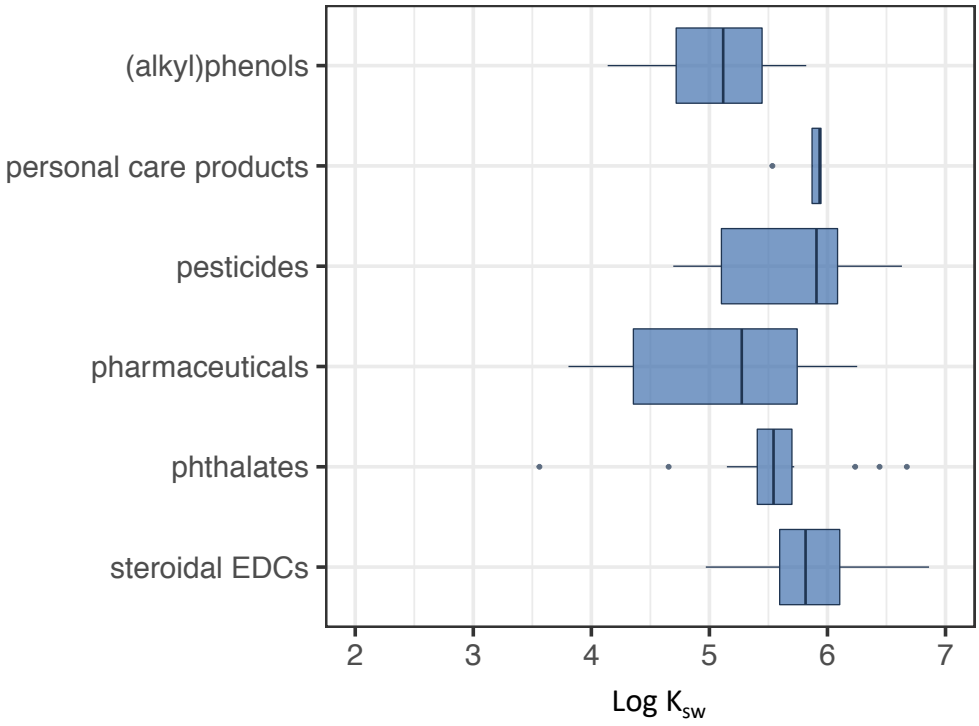


Figure 3. The calculated sorbent-water equilibrium partitioning coefficients (Log K_{sw}) of the different classes, i.e. alkylphenols (n=4), personal care products (n=5), pesticides (n=25), pharmaceuticals (n=32), phthalates (n=15) and steroidal EDCs (n=50). The batch systems were kept at a constant temperature of 8°C, pH of 7 and salinity of 35 psu.

In line with the ever-increasing number of chemicals that is released into the environment [1], several passive sampling-based studies have attempted to mathematically model log K_{sw} using various physico-chemical properties of the compounds under investigation as input [7,34–36]. Up until now, log P and molecular weight (MW) have been correlated frequently to the log K_{sw} (r^2=0.92, n=65) [7,34,35]. However, using our complete dataset, lack-of-fit (p-value < 0.05) between log P or MW and log K_{sw} was observed. Smedes et al. (2018) observed similar findings for the PAHs (polycyclic aromatic hydrocarbons), PCBs (polychlorinated biphenyls) and phthalates [37]. Therefore, we evaluated the modelling potential of a number of other physico-chemical properties, including V_x (molecular volume), qA^- (most negative charge on O, N, S, X atoms), H_y (hydrophilic factor), vapor pressure, bioconcentration factor, number of carbon atoms, pKa, water solubility and polar surface area. No valid model (R^2=0.18, n=115, all compounds for which described physico-chemical parameters were available) was however obtained for predicting log K_{sw} with none of the above-mentioned parameters or combinations thereof using the complete dataset.

3.2.3 Do increasing organic contaminant concentrations affect K_{sw}?

The influence of dissolved organic contaminant concentrations on their partitioning between artificial seawater and hydrophilic DVB was investigated by varying compound concentrations while keeping the amount of sorbent constant, as depicted for a selection of compounds in Figure 4. Changes in partitioning and surface heterogeneity (reflected by the Freundlich exponent, n) were assessed by calculating the K_{sw} (Eq. 1) and Freundlich isotherms (Eq. 2) respectively (Table C3). For the steroidal EDCs, higher dissolved concentrations resulted in increased K_{sw} (n>1), showing that higher initial steroidal EDC concentrations tend to enhance sorption capacity. This phenomenon has also been reported for methylene blue and phenol [38–41], bearing a similar cyclic aromatic structure as do the steroidal EDCs. For the (alkyl)phenols, personal care products, pesticides, pharmaceuticals and phthalates, dissolved concentrations exceeding the linear ranges of the isotherms resulted for 95% of the 131 studied compounds in reduced K_{sw} (n<1). The latter indicates that partitioning for these groups

of organic contaminant depends merely on the available hydrophilic DVB sorption sites. The aforementioned findings stress the need to examine the linear range of the partitioning isotherms for the different organic contaminants. For more than 75% of the steroidal EDCs (n>1) and all the other classes of studied organic contaminants (n<1), linear isotherms were observed when C_e was, respectively, lower than 8.5 ng L^{-1} and 70 ng L^{-1}. The C_e-levels applied in our experimental set-up are in the same order of magnitude than seawater concentrations recently measured in the Belgian Part of the North Sea for the contaminants studied [13,23,24].

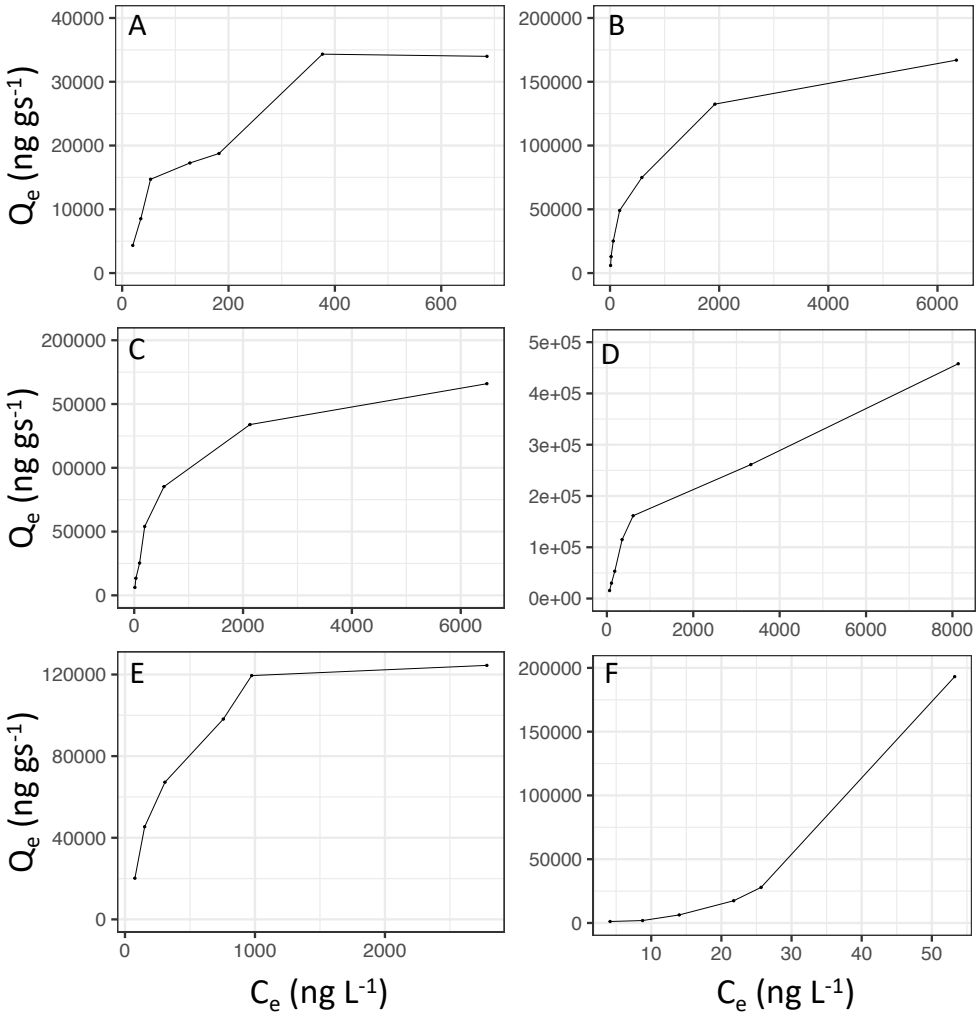


Figure 4. Partitioning isotherms (T = 8°C) for a set of selected compounds representing the 6 different classes, i.e. (a) (alkyl)phenols: isopropylphenol, (b) personal care products: methylparaben, (c) pesticides: acetamiprid, (d) pharmaceuticals: bezafibrate, (e) phthalates: monomethyl phthalate, and (f) steroidal EDCs: 11β-hydroxyandrosterone.

3.2.4 Do environmental conditions affect K_{sw}?

The dependency of the K_{sw} on environmental conditions was investigated using the Box-Behnken RSM design. Temperature, pH and salinity significantly ($p < 0.05$) impacted the K_{sw} for most of the contaminants. Only for the phthalates and alkylphenols (Tables C4 and C5), temperature did not significantly affect K_{sw}. Overall, the effect of solely temperature on the K_{sw} was more significant as compared to pH and salinity.

To better understand the nature of the partitioning process upon changing temperature, a number of thermodynamic parameters (Figure 5 and Table C6) were calculated using the van't Hoff equation (Eq. 3) and the change in Gibbs free energy (Eq. 4). For all compounds (at fixed conditions of pH and salinity), a decrease in log K_{sw}-values was observed (difference in log K_{sw} ranged between 0.06 and 5.19), when increasing temperature with 12°C (based on environmental range) confirming the exothermic nature ($\Delta H < 0$) of the partitioning process. The magnitude of ΔH indicates the nature of the sorption process; i.e. between 0 and -20 kJ mol^{-1} for physisorption and lower than -100 kJ mol^{-1} for chemisorption [42,43]. It should be noted that there is no sharp boundary between physisorption and chemisorption [44]. The largest negative enthalpies were observed for the steroidal EDCs ($\overline{\Delta H}$ = -114 kJ mol^{-1}), followed by $\overline{\Delta H}_{phthalates}$ = -88 kJ mol^{-1}, $\overline{\Delta H}_{alkylphenols}$ = -56 kJ mol^{-1}, $\overline{\Delta H}_{personal\ care\ products}$ = -26 kJ mol^{-1}, $\overline{\Delta H}_{pesticides}$ = -25 kJ mol^{-1} and $\overline{\Delta H}_{pharmaceuticals}$ = -14 kJ mol^{-1}.

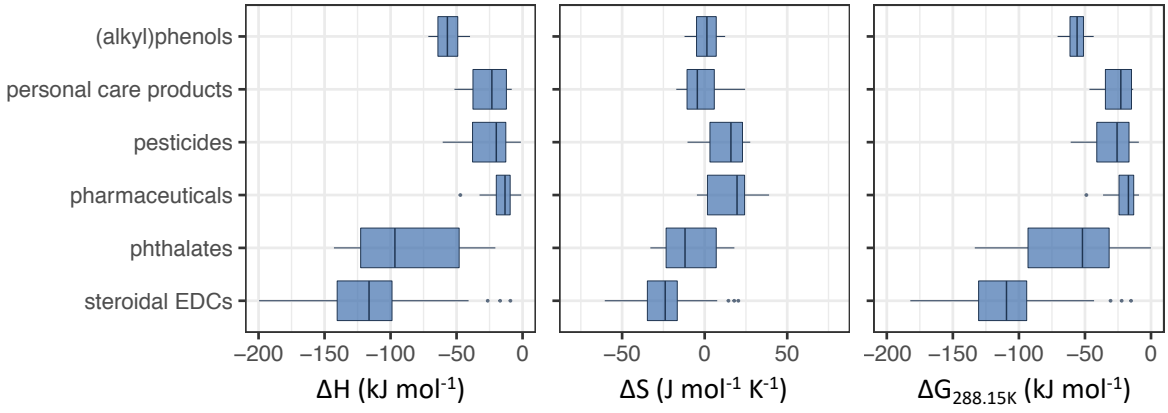


Figure 5. Thermodynamic parameters, i.e. change in enthalpy (ΔH), change in entropy (ΔS) and change in free Gibbs energy (ΔG) during sorption for the alkylphenols, personal care products, pesticides, pharmaceuticals, phthalates and steroidal EDCs.

Physisorption indicates to be the main driver for partitioning of personal care products, pesticides, pharmaceuticals and alkylphenols, and is dominated by Van der Waals interactions. The sorption of phthalates and steroidal EDCs was shown to be driven by a combination of physisorption and chemisorption (covalent interactions). For those compounds for which partitioning is mainly dominated by physisorption, the sorption process is more reversible. This could be useful to perform e.g. passive dosing experiments for toxicity testing [45].

The steroidal EDCs also demonstrated a negative ΔS ($\overline{\Delta S}_{steroidal\ EDCs}$ = - 23 J mol^{-1} K^{-1}), suggesting no significant alteration of the molecular structure in the sorbed state [46,47], which implies that the sorption process is enthalpy-driven. For the other compound classes, less negative ΔH and mainly positive ΔS values were observed. Vinmonses et al. (2009) suggested that positive ΔS values mark structural changes taking place on the sorbent, and as such increase randomized binding during sorption [48]. The magnitude of the negative $\Delta G_{288.15K}$ value indicates the spontaneous nature of the equilibrium sorption process, with higher negative values marking a more rapid and spontaneous sorption at lower temperatures according to Ahmad and Kumar (2010) and Vimonses et al. (2009, while Kebede (2013) report stronger bonding at negative $\Delta G_{288.15K}$ values. Our negative $\Delta G_{288.15K}$ data for the steroidal EDCs thus indicate a more rapid, spontaneous and stronger bonding of the latter to the sorbent [48–50], offering an explanation for their relatively high log K_{sw} as compared to the other compound classes (Figure 3).

With respect to the effect of pH, the log K_{sw} were not significantly (p-value > 0.05) impacted, except for 11 out of 131 organic contaminants. No general conclusion can however be drawn with relation to the impact of the pH on compound behaviour for all compound groups as the chemical speciation of organic molecules in water is governed by both the aqueous pH and the compounds' pKas. Similar results were reported by Stroski et al. (2018), with respect to the effect of pH on the sorption of 28 out of 31 pesticides and pharmaceuticals [51]. Only for strong cationic (rimantadine and trimethoprim) and strong anionic compounds (i.e.

sulfadoxine, sulfamethazine, sulfamethoxazole and clorfibric acid) a pH dependency was noticed. An increased pH caused lower (difference in log K_{sw} ranged between 0.24 and 0.82 over 3 pH-units) or higher (difference in log K_{sw} ranged between 0.14 and 0.22 over 3 pH units) K_{sw} for cationic and anionic compounds, respectively. These results are in line with the work of Jeong et al. (2017), which reported that pH tendancy can only be predicted for anionic and cationic compounds. In the current study, however, this was merely observed for strong cationic and anionic compounds, more specifically mecoprop, rimantadine, trimethoprim, venlafaxine, sulfadoxin, sulfamethazine, sulfamethoxazole and clofibric acid.

Different salt concentrations were used to assess the influence of ionic strength on partitioning. Altering the salinity did not impact (within the experimentally observed standard deviations of the RSM) the K_{sw} of the steroidal EDCs, phthalates and (alkyl)phenols ($p > 0.05$). For the personal care products, pesticides and pharmaceuticals, the influence of salinity on the K_{sw} was compound specific (difference in log K_{sw} ranged between 0.01 and 1.16 over 35 psu units). These findings agree well with previous work, in which it was demonstrated that the effect of salinity is compound-specific [21,52]. As salinity is known to impact the ionic composition of seawater, it may be anticipated that the sorption process, which is pKa-dependent is affected by the salinity in a compound-specific way. However, no significant relationship between the ionic strength and compound-specific log K_{sw} value of the 131 selected organic contaminants was observed for any of the sub-classes and the complete dataset.

4 CONCLUSIONS

For 131 emerging organic contaminants with a log P ranging from -1.3 to 9.85, K_{sw} describing the partitioning between artificial seawater and hydrophilic DVB, were determined. Combining measured K_{sw} and functional characteristics of the hydrophilic DVB sorbent (carboxylic and highly cross-linked divinylbenzene moieties) indicate the superior affinity of hydrophilic DVB for a broad polarity range of organic compounds as compared to any other sorbent described

so far. All studied emerging organic contaminants displayed a linear isotherm at environmentally relevant equilibrium water concentration ranges. At higher ranges, possible concentration effects were observed for the alkylphenols, personal care products, pesticides, pharmaceuticals and phthalates, while this was not the case for the steroidal EDCs. Nevertheless, at environmentally relevant concentrations, the sorption of a mixture of 131 micropollutants was sufficiently linear to provide quantitative results upon environmental application of the sorbent. Deeper investigation into the impact of altering environmental conditions demonstrated that the log K_{sw} mainly depended on temperature (log $K_{sw\ max,\ difference}$ = 5.19 over 12°C units), while pH (log $K_{sw\ max,\ difference}$ = 0.82 over 3 pH-units) and salinity (log $K_{sw\ max,\ difference}$ = 1.16 over 35 psu-units) were less important. The calculated thermodynamic parameters proved that the uptake of alkylphenols, personal care products, pesticides and pharmaceuticals was mainly dominated by physisorption, while the uptake of phthalates and steroidal EDCs was mediated by a combination of physisorption and chemisorption. This research offers new perspectives for both active and passive sampling studies, since it established that the hydrophilic DVB sorbent is promising for sampling a physico-chemically diverse range of organic contaminants from aqueous matrices.

REFERENCES

[1] M. Scheringer, Environmental chemistry and ecotoxicology: in greater demand than ever, Environ. Sci. Eur. 29 (2017) 3. doi:10.1186/s12302-016-0101-x.

[2] A. Andrade-Eiroa, M. Canle, V. Leroy-Cancellieri, V. Cerdà, Solid-phase extraction of organic compounds: A critical review (Part I), TrAC - Trends Anal. Chem. 80 (2016) 641–654. doi:10.1016/j.trac.2015.08.015.

[3] M. Asgarpour Khansary, S. Shirazian, M. Asadollahzadeh, Polymer-water partition coefficients in polymeric passive samplers, Environ. Sci. Pollut. Res. 24 (2017) 2627–2631. doi:10.1007/s11356-016-8029-7.

[4] J.M. Thompson, C.H. Hsieh, R.G. Luthy, Modeling uptake of hydrophobic organic contaminants into polyethylene passive samplers, Environ. Sci. Technol. 49 (2015) 2270–2277. doi:10.1021/es504442s.

[5] L. Ahrens, A. Daneshvar, A.E. Lau, J. Kreuger, Characterization of five passive sampling devices for monitoring of pesticides in water, J. Chromatogr. A. 1405 (2015) 1–11. doi:10.1016/j.chroma.2015.05.044.

[6] A. Martin, C. Margoum, J. Randon, M. Coquery, Silicone rubber selection for passive sampling of pesticides in water, Talanta. 160 (2016) 306–313. doi:10.1016/j.talanta.2016.07.019.

[7] F. Smedes, R.W. Geertsma, T. Van Der Zande, K. Booij, Polymer-water partition coefficients of hydrophobic compounds for passive sampling: Application of cosolvent models for validation, Environ. Sci. Technol. 43 (2009) 7047–7054. doi:10.1021/es9009376.

[8] S. Lissalde, A. Charriau, G. Poulier, N. Mazzella, R. Buzier, G. Guibaud, Overview of the Chemcatcher® for the passive sampling of various pollutants in aquatic environments Part B: Field handling and environmental applications for the monitoring of pollutants and their biological effects, Talanta. 148 (2016) 572–582. doi:10.1016/j.talanta.2015.06.076.

[9] A. Charriau, S. Lissalde, G. Poulier, N. Mazzella, R. Buzier, G. Guibaud, Overview of the Chemcatcher® for the passive sampling of various pollutants in aquatic environments Part A: Principles, calibration, preparation and analysis of the sampler, Talanta. 148 (2016) 556–571. doi:10.1016/J.TALANTA.2015.06.064.

[10] K. Booij, S. Chen, Review of atrazine sampling by polar organic chemical integrative samplers and Chemcatcher, Environ. Toxicol. Chem. 37 (2018) 1786–1798. doi:10.1002/etc.4160.

[11] D. a Alvarez, J.D. Petty, J.N. Huckins, T.L. Jones-Lepp, D.T. Getting, J.P. Goddard, et al., Development of a passive, in situ, integrative sampler for hydrophilic organic contaminants in aquatic environments., Environ. Toxicol. Chem. 23 (2004) 1640–1648. doi:10.1897/03-603.

[12] C. Metcalfe, M.E. Hoque, T. Sultana, C. Murray, P. Helm, S. Kleywegt, Monitoring for contaminants of emerging concern in drinking water using POCIS passive samplers, Environ. Sci. Process. Impacts. 16 (2014) 473. doi:10.1039/c3em00508a.

[13] S. Huysman, L. Van Meulebroek, F. Vanryckeghem, H. Van Langenhove, K. Demeestere, L. Vanhaecke, Development and validation of an ultra-high performance liquid chromatographic high resolution Q-Orbitrap mass spectrometric method for the simultaneous determination of steroidal endocrine disrupting compounds in aquatic matrices, Anal. Chim. Acta. 984 (2017). doi:10.1016/j.aca.2017.07.001.

[14] N. Fontanals, R.M. Marcé, F. Borrull, Overview of the novel sorbents available in solid-phase extraction to improve the capacity and selectivity of analytical determinations, Contrib. to Sci. 6 (2011) 199–213. doi:10.2436/20.7010.01.97.

[15] European Commission, Commission Implementing Decision (EU) 2015/495 of 20 March 2015 establishing a watch list of substances for Union-wide monitoring in the field of water policy pursuant to Directive 2008/105/EC of the European Parliament and of the Council, 2015. doi:http://eur-

lex.europa.eu/pri/en/oj/dat/2003/l_285/l_28520031101en00330037.pdf.
[16] N.A. Slobodnik, NORMAN Position Paper Collection, exchange and interpretation of data on emerging substances Towards a harmonised approach for collection and interpretation of data on emerging substances in support of European environmental policies, 2014. www.norman-network.net (accessed December 4, 2018).
[17] Ospar, OSPAR Commission, Mar. Ecol. Prog. Ser. (2014). https://www.ospar.org/about/publications (accessed December 4, 2018).
[18] European Chemicals Agency, Understanding Reach, ECHA - Eur. Chem. Agency. (2015) 2015. https://echa.europa.eu/regulations/reach/understanding-reach%5Cnhttp://echa.europa.eu/web/guest/regulations/reach/understanding-reach.
[19] C. Copeland, Clean water act: A summary of the law, in: Atmos. Depos. Pollut. EPA, 2012. doi:10.4172/2332-0915.1000133.
[20] A. International, ASTM D-1141-98(2013) Seawater, 2013.
[21] S. Bayen, E. Segovia, L.L. Loh, D.F. Burger, H.S. Eikaas, B.C. Kelly, Application of Polar Organic Chemical Integrative Sampler (POCIS) to monitor emerging contaminants in tropical waters, Sci. Total Environ. 482–483 (2014) 15–22. doi:10.1016/j.scitotenv.2014.02.082.
[22] S.L. MacLeod, E.L. McClure, C.S. Wong, Laboratory calibration and field deployment of the polar organic chemical integrative sampler for pharmaceuticals and personal care products in wastewater and surface water, Environ. Toxicol. Chem. 26 (2007) 2517–2529. doi:10.1897/07-238.1.
[23] S. Huysman, L. Van Meulebroek, O. Janssens, F. Vanryckeghem, H. Van Langenhove, K. Demeestere, et al., Targeted quantification and untargeted screening of alkylphenols, bisphenol A and phthalates in aquatic matrices using ultra-high-performance liquid chromatography coupled to hybrid Q-Orbitrap mass spectrometry, Anal. Chim. Acta. 1049 (2019) 141–151. doi:10.1016/j.aca.2018.10.045.
[24] F. Vanryckeghem, S. Huysman, H. Van Langenhove, L. Vanhaecke, K. Demeestere, Multi-residue quantification and screening of emerging organic micropollutants in the Belgian Part of the North Sea by use of Speedisk extraction and Q-Orbitrap HRMS (submitted), Mar. Pollut. Bull. (2019).
[25] C. Harman, I.J. Allan, E.L.M. Vermeirssen, Calibration and use of the polar organic chemical integrative sampler-a critical review, Environ. Toxicol. Chem. 31 (2012) 2724–2738. doi:10.1002/etc.2011.
[26] H.N. Tran, S.J. You, A. Hosseini-Bandegharaei, H.P. Chao, Mistakes and inconsistencies regarding adsorption of contaminants from aqueous solutions: A critical review, Water Res. 120 (2017) 88–116. doi:10.1016/j.watres.2017.04.014.
[27] R. Kizil, J. Irudayaraj, K. Seetharaman, Characterization of irradiated starches by using FT-Raman and FTIR spectroscopy, J. Agric. Food Chem. 50 (2002) 3912–3918. doi:10.1021/jf011652p.
[28] X. Guo, L. Liu, J. Wu, J. Fan, Y. Wu, Qualitatively and quantitatively characterizing water adsorption of a cellulose nanofiber film using micro-FTIR spectroscopy, RSC Adv. 8 (2018) 4214–4220. doi:10.1039/c7ra09894d.
[29] M. Mizuguchi, M. Nara, K. Kawano, K. Nitta, FT-XR study of the Ca2+-binding to bovine α-lactalbumin. Relationships between the type of coordination and characteristics of the bands due to the Asp COO-groups in the Ca2+-binding site, FEBS Lett. 417 (1997) 153–156. doi:10.1016/S0014-5793(97)01274-X.
[30] Y. Taguchi, T. Noguchi, Drastic changes in the ligand structure of the oxygen-evolving Mn cluster upon Ca2+ depletion as revealed by FTIR difference spectroscopy, Biochim. Biophys. Acta - Bioenerg. 1767 (2007) 535–540. doi:10.1016/j.bbabio.2006.11.002.
[31] B. Davidson, A.A. Murray, A. Elfick, N. Spears, Raman Micro-Spectroscopy Can Be Used to Investigate the Developmental Stage of the Mouse Oocyte, PLoS One. 8 (2013) e67972. doi:10.1371/journal.pone.0067972.
[32] Y. Jeong, A. Schäffer, K. Smith, Comparison of the sampling rates and partitioning behaviour of polar and non-polar contaminants in the polar organic chemical integrative sampler and a monophasic mixed polymer sampler for application as an equilibrium

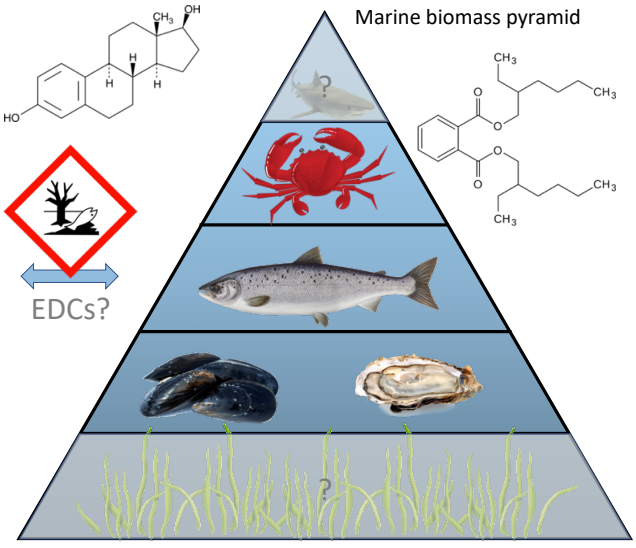
passive sampler, Sci. Total Environ. 627 (2018) 905–915. doi:10.1016/j.scitotenv.2018.01.273.
[33] Y. Jeong, A. Schäffer, K. Smith, Equilibrium partitioning of organic compounds to OASIS HLB, as a function of compound concentration, pH, temperature and salinity, Chemosphere. 174 (2017) 297–305. doi:10.1016/j.chemosphere.2017.01.116.
[34] R. Lohmann, Critical review of low-density polyethylene's partitioning and diffusion coefficients for trace organic contaminants and implications for its use as a passive sampler, Environ. Sci. Technol. 46 (2012) 606–618. doi:10.1021/es202702y.
[35] Y. Choi, Y.M. Cho, R.G. Luthy, Polyethylene-water partitioning coefficients for parent- and alkylated-polycyclic aromatic hydrocarbons and polychlorinated biphenyls, Environ. Sci. Technol. 47 (2013) 6943–6950. doi:10.1021/es304566v.
[36] H. Liu, M. Wei, X. Yang, C. Yin, X. He, Development of TLSER model and QSAR model for predicting partition coefficients of hydrophobic organic chemicals between low density polyethylene film and water, Sci. Total Environ. 574 (2017) 1371–1378. doi:10.1016/J.SCITOTENV.2016.08.051.
[37] F. Smedes, Silicone–water partition coefficients determined by cosolvent method for chlorinated pesticides, musks, organo phosphates, phthalates and more, Chemosphere. 210 (2018) 662–671. doi:10.1016/j.chemosphere.2018.07.054.
[38] O. V. Ovchinnikov, A. V. Evtukhova, T.S. Kondratenko, M.S. Smirnov, V.Y. Khokhlov, O. V. Erina, Manifestation of intermolecular interactions in FTIR spectra of methylene blue molecules, Vib. Spectrosc. 86 (2016) 181–189. doi:10.1016/J.VIBSPEC.2016.06.016.
[39] O. Abdelwahab, N.K. Amin, Adsorption of phenol from aqueous solutions by Luffa cylindrica fibers: Kinetics, isotherm and thermodynamic studies, Egypt. J. Aquat. Res. 39 (2013) 215–223. doi:10.1016/j.ejar.2013.12.011.
[40] R. Ocampo-Perez, R. Leyva-Ramos, J. Mendoza-Barron, R.M. Guerrero-Coronado, Adsorption rate of phenol from aqueous solution onto organobentonite: Surface diffusion and kinetic models, J. Colloid Interface Sci. 364 (2011) 195–204. doi:10.1016/j.jcis.2011.08.032.
[41] G. Lü, J. Hao, L. Liu, H. Ma, Q. Fang, L. Wu, et al., The adsorption of phenol by lignite activated carbon, Chinese J. Chem. Eng. 19 (2011) 380–385. doi:10.1016/S1004-9541(09)60224-X.
[42] R.C. Ahlert, Process dynamics in environmental systems, Environ. Prog. 16 (1997) S6–S7. doi:10.1002/ep.3300160107.
[43] M.C. Leuenberger, M.F. Schibig, P. Nyfeler, Gas adsorption and desorption effects on cylinders and their importance for long-term gas records, Atmos. Meas. Tech. 8 (2015) 5289–5299. doi:10.5194/amt-8-5289-2015.
[44] A.W. Adamson, Physical Chemistry of Surfaces, J. Electrochem. Soc. 124 (1977) 192C. doi:10.1149/1.2133374.
[45] K. Vorkamp, J.C. McGeer, How to Improve Exposure Assessment, in: Aquat. Ecotoxicol. Adv. Tools Deal. with Emerg. Risks, Academic Press, 2015: pp. 77–102. doi:10.1016/B978-0-12-800949-9.00004-8.
[46] M. Tejraj, B. Shivaputrappa, Kinetic and thermodynamic study on the sorption of liquids by polymer films: A simple laboratory experiment, 68 (1991) 343–346. doi:10.1021/ed068p343.
[47] M.G. Sujana, H.K. Pradhan, S. Anand, Studies on sorption of some geomaterials for fluoride removal from aqueous solutions, J. Hazard. Mater. 161 (2009) 120–125. doi:10.1016/j.jhazmat.2008.03.062.
[48] V. Vimonses, S. Lei, B. Jin, C.W.K. Chow, C. Saint, Kinetic study and equilibrium isotherm analysis of Congo Red adsorption by clay materials, Chem. Eng. J. 148 (2009) 354–364. doi:10.1016/j.cej.2008.09.009.
[49] S. Kebede, Groundwater in Ethiopia: Features, numbers and opportunities, 2013. doi:10.1007/978-3-642-30391-3.
[50] R. Ahmad, R. Kumar, Adsorptive removal of congo red dye from aqueous solution using bael shell carbon, Appl. Surf. Sci. 257 (2010) 1628–1633.

doi:10.1016/j.apsusc.2010.08.111.
[51] K.M. Stroski, J.K. Challis, C.S. Wong, The influence of pH on sampler uptake for an improved configuration of the organic-diffusive gradients in thin films passive sampler, Anal. Chim. Acta. 1018 (2018) 45–53. doi:10.1016/j.aca.2018.02.074.
[52] Z. Zhang, A. Hibberd, J.L. Zhou, Analysis of emerging contaminants in sewage effluent and river water: Comparison between spot and passive sampling, Anal. Chim. Acta. 607 (2008) 37–44. doi:10.1016/j.aca.2007.11.024.

IV

Field study - Active and passive sampling based approaches for monitoring endocrine disrupting compounds in the Belgian Part of the North Sea between 2016 and 2018

Active & passive sampling



Risk assessment

Adapted from:

(1) Huysman S, Vanryckegem F, Moeris S, A.C. De Schamphelaere K, Demestere K, Vanhaecke L. – in preparation

ABSTRACT

In recent years, endocrine disrupting compounds (EDCs) and in particular steroids, plastics additives and plasticizers have received an increasing societal and scientific interest because of their extensive use and potential biological activity. Nevertheless, data on the prevalence of these EDCs in the marine environment are scarce. This study presents a complementary approach for evaluating the EDC contamination status of the Belgian Part of the North Sea (BPNS), by employing active and passive sampling and UHPLC-HRMS analysis. In total, 97 EDCs were measured at 4 locations in the BPNS between 2016 and 2018, of which 66±9 compounds were detected during each sampling campaign. Thereby, 61±11% and 47±17% of the EDCs were detected by active and passive sampling, respectively, with an overlapping coverage of 31±14%.

The most frequently detected class of steroidal EDCs comprised the corticosteroids with concentrations ranging between 8 and 104 ng L^{-1}. For the plastics additives and plasticizers, 4-ethylphenol (0.007 – 6.5 µg L^{-1}), dibutyl phthalate (0.011 – 5.3 µg L^{-1}) and di-ethylhexyl phthalate (1.3 - 504 ng L^{-1}) occurred at the highest concentrations. Subsequently, the measured EDC concentrations were compared to available environmental quality standards (EQS). The highest risk quotients were observed for 17β-estradiol, while lower risk quotients were noted for diethylhexyl phthalate (DEHP).

1 INTRODUCTION

A plethora of adverse effects have been reported, even at low concentrations (ng L^{-1}), upon exposure of aquatic organisms to endocrine disrupting compounds (EDCs) [1–4]. Steroids, plasticizers and plastics additives are amongst the most important EDC groups that have received increasing interest in recent years because of their extensive use and proclaimed biological activities. Indeed, it has been demonstrated that androgens cause masculinization in fish, e.g. zebrafish and mosquitofish [5,6], while oestrogens and progestagens significantly affect fish physiology - e.g. Atlantic salmon and sea bass - and may as such compromise reproductive development [1,2,7–10]. Another study demonstrated that corticosteroids disturb embryonic behaviour of zebrafish [11], whereas Bisphenol A showed to cause developmental and reproductive effects in zebra fish, frogs and swordtail fish [12,13]. Finally, also for the phthalates, toxic effects were observed in fish, amphibians and invertebrates, ranging from the disruption of genetics, hormone regulation, morphology to developmental defects [14]. The breath of adverse effects that has been noted for the above-mentioned EDCs on water-borne organisms urges in depth research into the monitoring of EDCs in the aquatic environment, which must be preceded by the development of appropriate sampling and analytical tools [15].

Highly sensitive and reliable analytical instrumental methods are needed for the analysis of a wide range of trace organic contaminants in aqueous matrices. The majority of the instrumental methods developed so far have been established using liquid chromatography (LC) coupled to tandem mass spectrometry (LC-MS/MS), particularly using triple quadrupole (QqQ) analysers [16,17]. (Ultra)high-performance liquid chromatography (U)-(HPLC) has shown its merits in meeting the demand for an efficient separation of a broad range of residues in aquatic matrices [18]. Furthermore, shifting from MS/MS to high-resolution mass spectrometry (HRMS) instruments, providing high-quality throughput data, high mass accuracy and fast scanning speeds,

has increased the number of compounds that can be analysed in 1 run to virtually unlimited [19]. Consequently, these analytical instrumental improvements have resulted in an increased sensitivity and selectivity, enabling the accurate quantification of a wide range of trace organic contaminants in aqueous matrices [20–22]. Despite of the considerable advancements on the instrumental side, the initial sampling and sample preparation steps that precede the analytical determination, remain crucial for the interpretation and biological relevance of the obtained concentrations. Conventional sampling techniques, i.e. active (grab) sampling, are limited to a specific time and place [23], imposing multiple sampling times to collect representative samples. In addition, monitoring studies using active sampling are frequently hindered by lack in sensitivity. This may be overcome by collecting large volume samples, of several up to 100 L, evidently at the expense of practicality and economics [24]. Amongst other available sampling techniques, passive sampling (applying a sampling device for a certain period of time directly in the aquatic environment) has surfaced as a promising tool, as it allows to extract and preconcentrate a wide range of compounds during exposure and provide more information on the bioavailable fractions. Passive sampling also reduces the required number of samples to be analysed and as such limits the total analysis cost [25].

A vast amount of studies have been published reporting on the prevalence of EDCs in freshwater bodies, i.e. surface, ground, drinking, and wastewater, with concentrations ranging within the ng until μg per liter range [26–30]. In this context, oestrogens and di-phthalates have been frequently reported, while data on androgens, progestagens, corticosteroids and mono-phthalates are more scarce. Scarcity of monitoring data is certainly an issue when it comes to the marine environment. Nevertheless, aquatic organisms are particularly susceptible to EDCs, because their entire life cycle is spent in continuous contact with the water [31]. Consequently, EDCs may accumulate in aquatic organisms throughout different trophic levels. Even more, biomagnification of EDCs may occur through the food chain and as such harmful concentrations (0.02 –

2.5 ng L^{-1}) may be reached in humans [32,33]. This stresses the need for monitoring EDC residues in the marine environment, which is also reflected by growing public and scientific concerns [34].

This work aimed at comparing the above-described sampling strategies, i.e. active and passive sampling, for monitoring EDCs in the marine environment of the Belgian Part of the North Sea (BPNS). To this end, 97 EDCs were analysed using our in-house optimized and successfully validated UHPLC-HRMS-based methods [21,35]. The contamination status of the BPNS is generally considered as high, as it is located near the English Channel, being one of the busiest seaways and affected marine ecosystems worldwide [36,37]. Water samples were collected along the BPNS during 4 sampling campaigns between 2016 and 2018. Complementarity of the active and passive sampling tools is discussed, and detected EDCs concentrations in the BPNS were compared to available environmental quality standards (EQS) for estimating the risk.

2 MATERIALS AND METHODS

2.1 Chemicals and reagents

In this study, 97 target compounds were considered, which were purchased from Accustandard (New Have, CT, USA) and Sigma Aldrich (St. Louis, MO, USA). The target compounds were selected based on relevant literature [38–47] and covered 6 classes, i.e. 33 androgens, 14 oestrogens, 12 progestagens, 11 corticosteroids, 5 (alkyl)phenols and 20 phthalates. The selected internal deuterated standards comprised 6 androgens, 5 oestrogens, 4 progestagens, 2 corticosteroids, 2 phenols, and 2 phthalates. Primary stock solutions and mixed standards, reaching concentrations between 1 and 1000 ng μL^{-1}, were prepared in Optima grade acetonitrile (Fisher scientific, Loughborough, UK). The solutions were stored in amber glass bottles at -20°C. Organic solvents were of Optima UHPLC-MS grade, purchased

at Fisher Scientific (Loughborough, UK). Reference seawater was prepared according to ASTM D-1141 [48] using inorganic salts supplied by Sigma Aldrich (St. Louis, MO, USA), i.e. NaCl, Na_2SO_4, MgCl.6(H_2O), $CaCl_2$.2(H_2O), $SrCl_2$.6(H_2O), KCl, $NaHCO_3$, KBr, H_3BO_3 and NaF. Ultrapure water was obtained by usage of a purified-water system (Millipore, Sartorius, Germany).

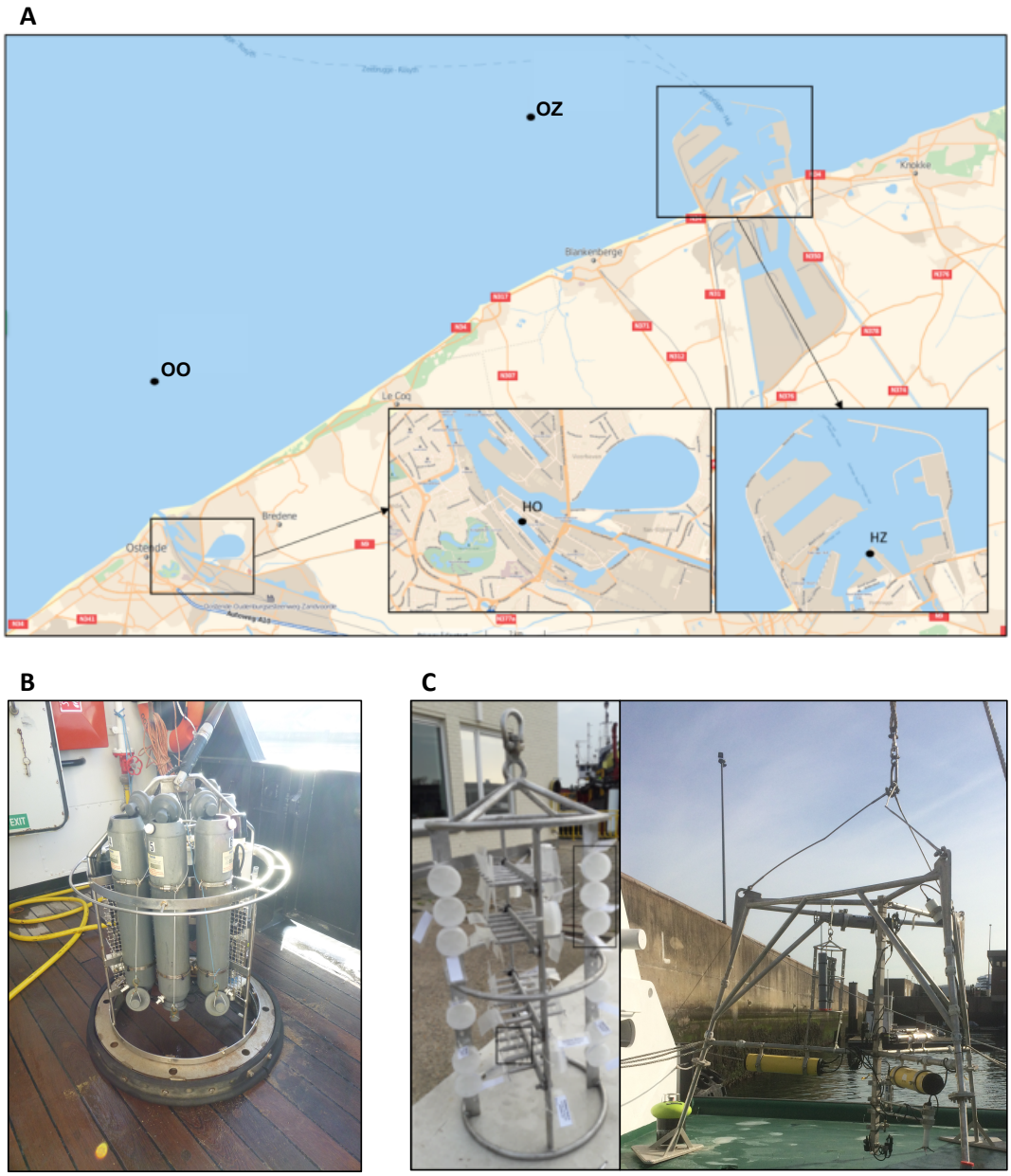


Figure 1. A represents the 4 sampling locations that were monitored in the BPNS, i.e. 51°21'37.78"N; 3° 6'49.01"O (OZ), 51°20'25.68"N; 3°12'12.11"O (HZ), 51°14'48.59"N; 2°55'39.61"O (OO) and 51°13'34.68"N; 2°56'8.00"O (HO). B depicts the Niskin bottles for taking active samples. C indicates how the passive samplers were attached to the tripod.

2.2 Study area and sampling

Sampling was performed in the BPNS, which is located near the English Channel. An overview of the study area and the sampling stations is depicted in Figure 1. Sampling of the target compounds was carried out at four locations, i.e. 51.22263°, 2.9357° (HO, harbour Oostende); 51.340073°, 3.203393° (HZ, harbour Zeebrugge); 51.24683°, 3.113615° (OO, open sea Oostende); and 51.360494°, 3.113615° (OZ, open sea Zeebrugge). All sampling stations at open sea are located in the active fishing zone [49]. Locations at the harbours were representative for their major freshwater inputs, whereas the locations at open sea were selected in front of the harbours. The harbour stations were sampled directly from the quay, while off-shore stations in the North Sea were sampled with the larger research vessel Belgica. Depending on vessel availability and weather conditions, four sampling campaigns were carried out: November 2016 – February 2017, April – July 2017, October – December 2017 and March – May 2018 (Table 1). At the beginning of each sampling campaign, generally 3 grab samples (active sampling) were taken and 6 passive samplers were deployed. At the retrieval of the passive samplers, generally 3 grab samples were taken as well. Detailed information on the different sampling locations and type of samplers is depicted in Figure 1, and detailed information on the sampling campaigns can be consulted in Table 1.

More specifically, grab samples were taken at a depth of 3 m, using Niskin bottles [50]. For the analysis of the steroidal EDCs, (alkyl)phenols and phthalates, a total volume of 9 L was needed, comprising 3 replicates. Grab samples were collected in amber glass bottles, previously washed with methanol and ultrapure water. Upon arrival in the lab, grab samples were filtered (Glass Microfibre Filters Whatman™, 0.45 µm, 90 x 90 mm), acidified to pH 3 using 1 M HCl, and stored at 4°C prior to extraction.

For passive sampling, H_2O-philic DVB Speedisks™ were used. The latter consist of a polytetrafluoroethylene housing enclosing 400 mg hydrophilic DVB sorbent between

glass fibre membranes. Prior to deployment, passive samplers were precleaned and conditioned with 20 mL of 5% acetonitrile and 20 mL of ultrapure water, and stored in ultrapure water. Exposure time was roughly 2 months, details can be consulted in Table 1. Passive samplers were all attached in the same direction to a tripod that was attached on a buoy for assuring continuous exposure at an identical depth. At the end of exposure, each sampler was rinsed with ultra-pure water, wrapped into aluminium foil, and stored weatably at 4°C until analysis. Analyses were performed within 3 days upon arrival of the samplers at the lab.

Table 1. An overview of the different sampling campaigns (SC). During deployment (D) and retrieval (R) of passive samplers (P), active samples were taken. The symbols ✓ and X correspond to the availability or absence of samples, respectively. NA (not available) indicates that no data are available because the ship broke down at the time for retrieval.

SC	Location	Start	End	D	R	P
1	OZ	23/11/2016	06/02/2017	✓	✓	✓
	HZ	25/11/2016	02/02/2017	✓	✓	✓
	HO	25/11/2016	02/02/2017	✓	✓	✓
2	OZ	23/5/2017	14/07/2017	✓	✓	✓
	HZ	13/4/2017	20/6/2017	✓	X	X
	OO	2/5/2017	26/7/2017	✓	✓	✓
	HO	13/4/2017	20/6/2017	✓	✓	✓
3	OZ	26/10/2017	19/12/2017	✓	✓	✓
	HZ	16/10/2017	18/12/2017	✓	✓	✓
	OO	26/10/2017	10/4/2018	✓	✓	X
	HO	16/10/2017	18/12/2017	✓	✓	✓
4	OZ	29/3/2018	NA	✓	X	X
	HZ	29/3/2018	17/5/2018	✓	✓	✓
	OO	29/3/2018	NA	✓	X	X
	HO	29/3/2018	17/5/2018	✓	✓	✓

2.3 Analytical procedures

2.3.1 Extraction

2.3.1.1 Active samples

Prior to extraction, samples were brought to room temperature. Afterwards, samples were spiked with a mixture of deuterated internal standards, i.e. 10 ng L^{-1} for the deuterated steroidal EDCs, 100 ng L^{-1} for the deuterated phthalates, and 400 ng L^{-1} for the deuterated phenols. EDCs were extracted according to two previously published protocols, for the steroidal EDCs [21] and the (alkyl)-phenols and phthalates [35], respectively.

For the steroidal EDCs, 2.5 L of sample was adjusted to pH 7 using 1M NaOH. Subsequently, H_2O-philic DVB sorbents used for solid-phase extraction were conditioned with 20 mL of 5% acetonitrile and 20 mL of ultrapure water under vacuum. Next, samples (2.5 L) were drawn through H_2O-philic DVB Speedisks under vacuum, followed by a washing step with 20 mL of ultrapure water, upon which a vacuum was applied on the Speedisks to remove residual water drops. Afterwards, elution was performed by gravity using sequentially 5 mL of acetonitrile and 5 mL of acetonitrile acidified with 0.1% formic acid. The combined extracts were vaporized under a gentle stream of nitrogen at a temperature of 50°C until dry. The extract was reconstituted in 150 µL of methanol and ultrapure water (40/60, v/v), centrifuged at 2430 g, and the supernatant was transferred into an LC-MS glass vial prior to analysis.

For the (alkyl)phenols and phthalates, 0.5 L of sample was used and the pH was kept at 3. Subsequently, Oasis® HLB cartridges (6 cc, 500 mg sorbent, 60 µm particle size; Waters) were conditioned with 6 mL of 5 % acetonitrile diluted in ultrapure water and 7 mL of ultrapure water under vacuum. Next, samples (0.5 L) were drawn through the cartridges under vacuum (10 mL min^{-1}), followed by a washing step with 8 mL of ultrapure water and applying a vacuum (20 min) to remove residual water drops. Afterwards, elution was performed by using 9 mL of 0.1% formic acid in acetonitrile.

The extracts were vaporized under a mild stream of nitrogen at a temperature of 40°C until dry. Extracts were reconstituted in 150 µL of water/acetontrile (5/95, v/v) and centrifuged at 2430 g. Finally, supernatants were transferred into LC-MS vials prior to analysis.

2.3.1.2 Passive samplers

Passive samplers were cleaned from debris and mud with ultrapure water. This was followed by a washing step with 20 mL of ultrapure water, during which a vacuum was applied on the Speedisks. Elution was performed by gravity using sequentially 5 mL of acetonitrile and 5 mL of acetonitrile acidified with 0.1% formic acid. The extracts were combined for evaporation and divided in two parts, to allow both to be dried at a different temperature. Evaporation was performed at 50 °C for the steroidal EDCs and at 40°C for the (alkyl)phenols and phthalates. Extracts were reconstituted in 75 µL water/methanol (40/60, v/v) for the steroidal EDCs, and in 75 µL water/acetonitrile (5/95, v/v) for the (alkyl)phenols and phthalates.

2.3.2 Instrumental analysis

Quantification of EDCs in extracts obtained from active and passive samplers was performed using three in-house developed and validated UHPLC-HR-Q-Orbitrap™-MS methods. Separation was achieved using reversed phase chromatography with gradient elution using a Hypersil Gold column (1.9 µm, 100 x 2.1 mm). For the quantification of (alkyl)phenols and phthalates, an additional trap column was used for retarding any target contamination originating from the analytical instrument and eluent. Analyte detection was carried out on a Q-Exactive™ benchtop HRMS (Thermo Fisher Scientific, San-Francisco, USA). Details regarding the instrumental analysis have been published earlier, and can be consulted in Chapter II of this doctoral thesis [21,22].

2.4 Calculation of water concentration captured by passive samplers

For equilibrium samplers, the aqueous concentrations (C_w, g L^{-1}) are calculated from the accumulated amount of compounds by the passive sampler (N_s, g) divided by the sorbent-water partition coefficient (K_{sw}, L g^{-1}) and mass of the sampler (M_s, g) (see Eq 1) [51].

$$C_w = \frac{N_s}{K_{sw} M_s} \quad (1)$$

2.5 Ecological risk assessment

Based on the European Commission Technical Guidance document, the ecological risks of EDCs in water samples were assessed by using the risk quotient (RQ) method [52,53]. RQs were solely calculated for compounds for which an environmental quality standard (EQS) is available. This RQ can be calculated as the quotient of the measured environmental concentration (MEC) and the environmental quality standard (EQS) (see Eq 2).

RQ= MEC/EQS (2)

The exceedance of the RQ for any specific compound above 1 was considered as a risk, while an RQ below or equal to 1 was considered as no risk [54,55].

3 RESULTS AND DISCUSSION

3.1 Distribution of target compounds

The target compounds that were detected during the 4 sampling campaigns are semi-quantitatively depicted in Figure 2 for the steroidal EDCs, and in Figure 4 for the plastics additives and plasticizers. The full quantitative data can be consulted in Tables D1 – D14. All analysed EDC classes were ubiquitously present in the BPNS. The steroidal EDCs were mainly observed below 10 ng L^{-1} (except for corticosteroids) while the plastics additives and plasticizers were detected at concentrations ranging between 10 and 1000 ng L^{-1}.The most abundant class that was detected during the

different sampling campaigns and at the different locations comprised the corticosteroids. The natural (i.e. cortisone, cortisol and tetrahydrocortisone) and the synthetic corticosteroids (i.e. prednisone, prednisolone and dexamethasone) were the most prominently present. The maximal concentrations for the abovementioned corticosteroids were 39, 59, 69, 10E10, 8.0 and 21 ng L^{-1}, respectively. The unambiguous prevalence of these corticosteroids in the marine BPNS environment can be ascribed to their extensive use in human and veterinary medicine as therapeutic drugs for the treatment of various inflammatory and autoimmune diseases [56]. Different studies have observed that corticosteroids can be directly excreted or partially released in the aquatic environment following an incomplete elimination from wastewater treatment plants with removal efficiencies ranging between 73 and 99% [28,57,58]. Nevertheless, until now the prevalence of corticosteroids had only been reported in fresh water environments [57]. Therefore, this study is the first in its kind providing evidence that corticosteroids have already reached our marine environment. Another important finding pertains to the detection of 5α-dihydrotestosterone in all collected grab samples, while this compound was below the method's detection limit in the passive samples. This suggests that the DVB sampler is not able to capture the freely dissolved 5α-dihydrotestosterone fraction.

The prevalence of plastics additives and phthalates (Figure 3) was also clearly demonstrated in the BPNS. The analytes 4-ethylphenol, dibutyl phthalate (DBP) and di-ethylhexyl phthalate (DEHP) occurred at the highest concentrations in both active and passive samples, and this at every sampling location. The maximum detected concentrations for the above-mentioned plastics additives and phthalates amounted respectively 6.5, 5.3 and 0.7 µg L^{-1}. The occurrence of 4-ethylphenol can originate from wastewater treatment plant discharges and anthropogenic activities [59]. The high concentrations measured for DBP and DEHP may be caused by extensive industrial activities at the harbours or major freshwater inputs from land inwards.

Figure 2. Heat map depicting a semi-quantitative overview of the steroidal EDCs measured during the different sampling campaigns (SC) and at the 4 studied locations, i.e. open sea Zeebrugge (OZ), harbour Oostende (HO), harbour Zeebrugge (HZ) and open sea Oostende (OO). Concentrations that were detected below the MQL (method quantification limit) were calculated as MQL/2 for visualization. The grey shaded areas correspond to concentrations below the MDL (method detection limit). D and R refer to the concentration measured during the deployment (D) and retrieval (R) of active samples, while P refers to the concentration measured in passive samplers.

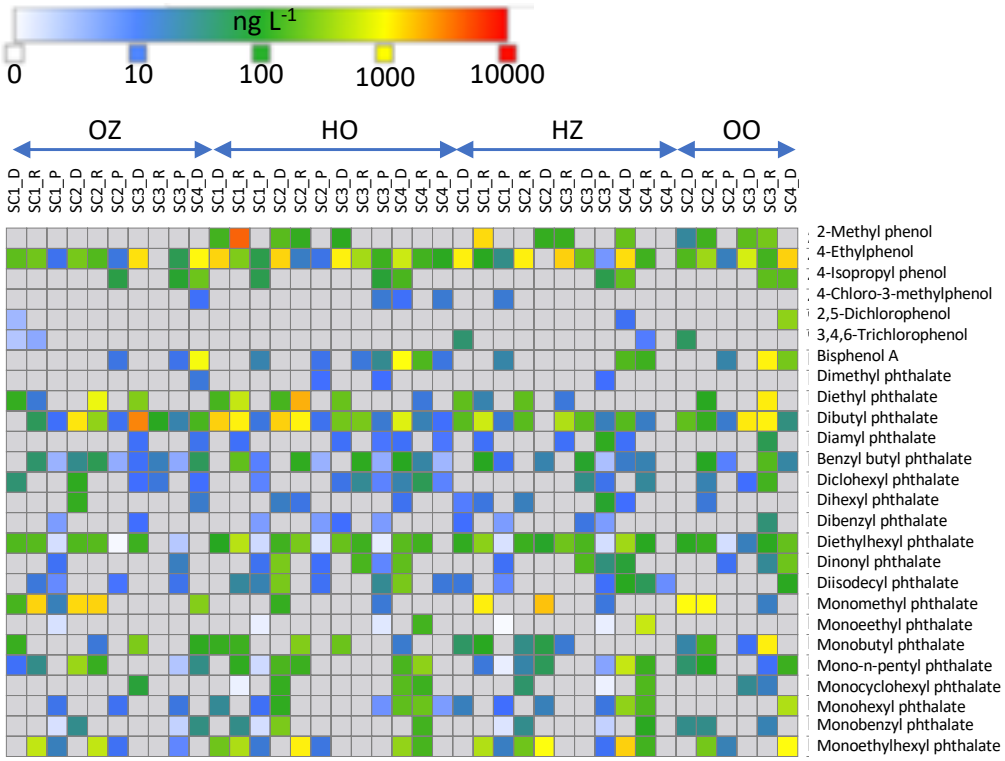


Figure 3. Heat map depicting a semi-quantitative overview of the plastics additives and plasticizers measured during the different sampling campaigns (SC) and at the 4 studied locations, i.e. open sea Zeebrugge (OZ), harbour Oostende (HO), harbour Zeebrugge (HZ) and open sea Oostende (OO). Concentrations that were detected below the MQL (method quantification limit) were calculated as MQL/2 for visualization. The grey shaded areas correspond to concentrations below the MDL (method detection limit). D and R refer to the concentration measured during the deployment (D) and retrieval (R) of active samples, while P refers to the concentration measured in passive samplers.

Interestingly, concentrations of DBP and DEHP measured at the harbours and open sea were of similar order of magnitude, confirming the widespread occurrence of the latter phthalates [60–62] and thus the anthropogenic pressure of these contaminants at the level of the active fishing zone [49].

Further, the primary (mono-)phthalate metabolites, monobutyl and mono-ethyl hexyl phthalate were ubiquitously present in almost every sampling campaign. This suggests that DBP and DEHP have been exposed to aquatic organisms and humans, as the primary metabolites are considered as relevant biomarkers for phthalate exposure [63,64]. Nevertheless, it should be noted that the described metabolic transformations-

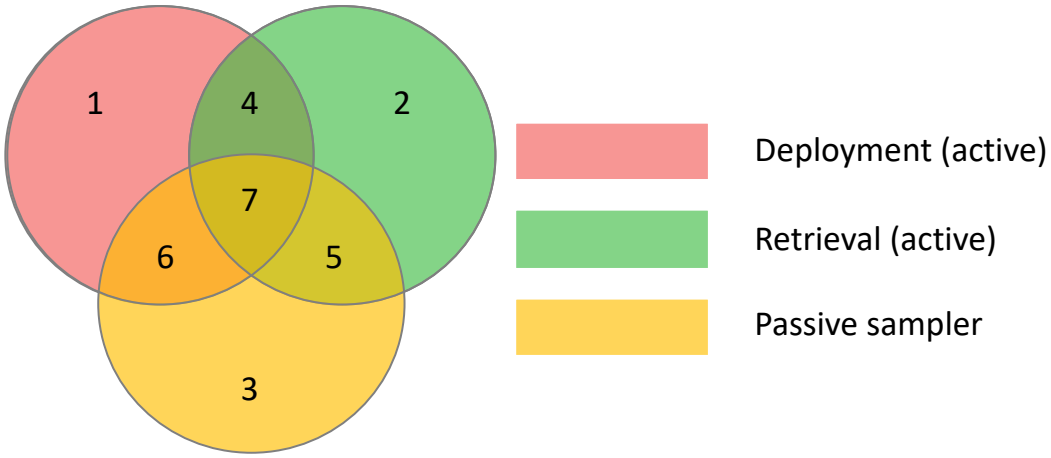
products are not limited to the above-mentioned primary (mono-)phthalates (Figure 3), but only the most abundant ones were here discussed.

3.2 Comparison of active and passive samples

The BPNS was monitored by using both an active and a passive sampling approach. The overlapping coverage of detected compounds between the different types of samplers is depicted in Figure 4. Similarity was only calculated for the sampling campaigns containing 1 passive and 2 active samplings (at deployment and retrieval). Similar coverage of the detected compounds was observed between the active samples that were taken during the same sampling campaign. Moreover, the average coverage of the active samples during the deployment and retrieval was 61±11% (n=11, with n number of complete sampling campaigns) and 60±12% (n=11) of the total number of detected compounds in the sampling campaign, respectively. However, an overlap of only 31±14% (n=11) of the compounds was observed between the active samples taken during the deployment and retrieval of each sampling campaign. This implies that the detected (number of) compounds changed over a period of 2 months.

When comparing the coverage of the above-mentioned two tested sampling strategies, it was observed that 61±11% and 47±17% of the detected EDCs were measured by active and passive sampling, respectively. Consequently, it can be concluded that the highest number of compounds was detected when using active sampling. Both types of sampling approaches demonstrated a similarity of 30±15% (n=11) of the detected compounds, indicating that both strategies measured a different fraction of the contaminants in the aquatic environment. Because of this, employing active and Speedisk passive sampling can be considered as complementary tools for evaluating the water quality and environmental contamination status. Furthermore, for the compounds that were detected using both approaches, lower concentrations were

obtained when using passive samples compared to active samples (except for 5α-dihydrotestosterone, testosterone acetate and equilin).



		Total Number of compounds	Intersection									
			1	2	3	4	5	6	7	D	R	P
HZ	SC1	61	9%	13%	7%	47%	4%	1%	18%	75%	82%	31%
	SC3	68	12%	11%	14%	9%	20%	8%	26%	55%	66%	68%
	SC4	68	18%	19%	23%	16%	10%	4%	11%	49%	56%	48%
OZ	SC1	59	29%	24%	18%	20%	2%	4%	4%	56%	49%	27%
	SC2	64	7%	10%	26%	8%	20%	5%	25%	44%	62%	75%
	SC3	74	43%	7%	10%	16%	3%	10%	10%	79%	37%	34%
HO	SC1	66	18%	10%	16%	29%	7%	3%	16%	66%	63%	43%
	SC2	68	9%	12%	19%	9%	17%	12%	22%	52%	60%	71%
	SC3	65	16%	8%	19%	36%	3%	3%	16%	70%	63%	41%
	SC4	80	15%	20%	15%	23%	7%	3%	18%	58%	68%	42%
OO	SC2	55	26%	18%	12%	17%	5%	12%	11%	65%	50%	39%

Figure 4. Venn-diagram depicting the number of compounds that were detected by active and passive sampling. During the deployment and retrieval of the passive (P; corresponding to the sections 3, 4, 5 and 7) samplers, active samples were taken; referred to as (D; corresponding to the sections 1, 4, 6 and 7) and (R; corresponding to the sections 2, 4, 5 and 7). The sampled locations were the harbour of Zeebrugge (HZ), Open sea Zeebrugge (OZ), harbour of Oostende (HO) and open sea Oostende (OO). Only the sampling campaigns (SC) of which the 3 measurement, i.e. D, R and P, were available are included.

Similar results have been reported by Terzopoulou et al. for 38 hydrophilic organic compounds, i.e. herbicides, pesticides, pharmaceuticals and phenols (2016) [65]. The active/passive concentration ratios are shown in Figure 5. The averaged ratio for the steroidal EDCs amounted 5.3. For the plastics additives and plasticizers, this averaged ratio amounted 31. Higher ratios were observed for the plastics additives and plasticizers than for the steroidal EDCs, as much higher concentrations were detected in the active samples for the former.

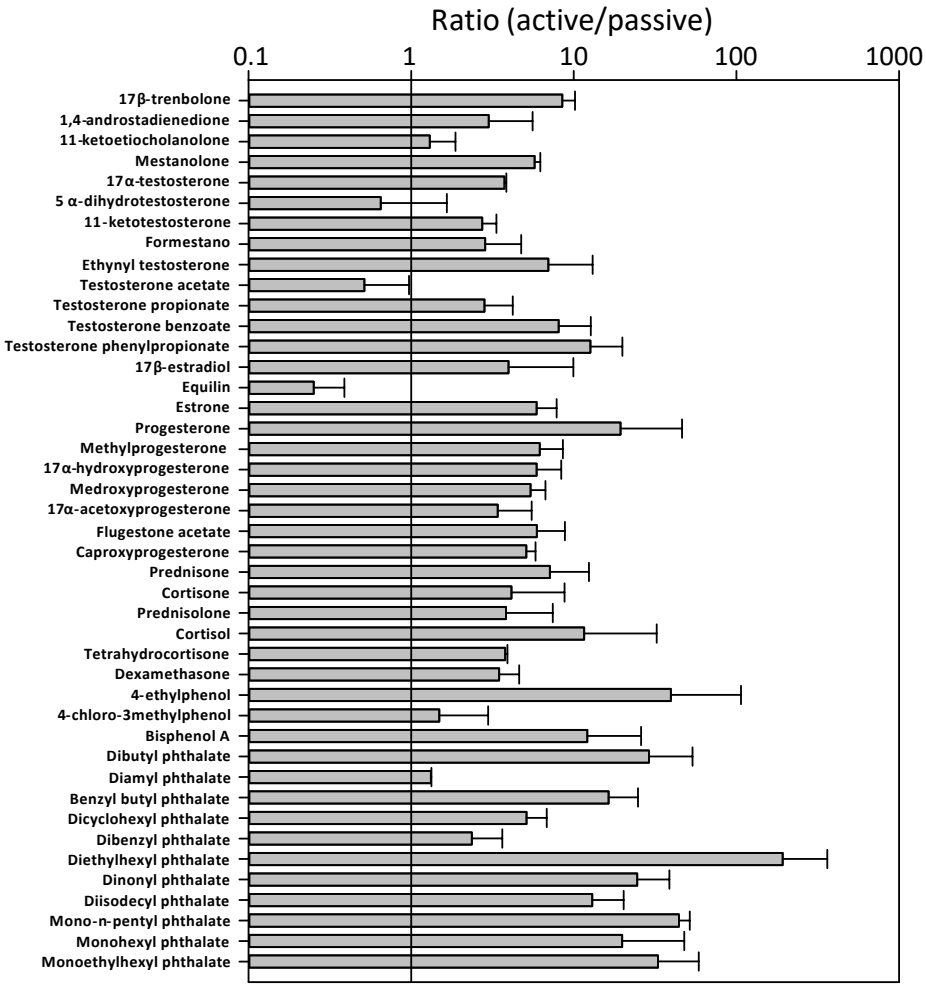


Figure 5. The average concentration ratios of the compounds that were detected in both active and passive samples (log scale for the x-axis). For the active concentrations, the average values that were obtained within the same sampling campaign were used.

3.3 Preliminary ecological risk assessment

The prevalence of the detected EDCs in the BPNS was further interpreted according to the RQ approach for those compounds for which an EQS was available. Existing or proposed EQS for marine waters of 17β-estradiol, 17α-ethinylestradiol and diethylhexyl phthalate (DEHP) are 0.08, 0.007 and 1300 ng L^{-1}, respectively [66–68]. The EQS of 17β-estradiol and 17α-ethinylestradiol used the aquatic ecosystem as the most sensitive protection goal and wasderived from a species sensitivity distribution of aquatic species. The EQS of DEHP used secondary poisoning of predators as the most sensitive protection goal and was derived using the$PNEC_{oral,\ mammal}$ (PNEC =

predicted no effect concentration of a mammalian predator via oral exposure) and a bioconcentration factor [68]. The RQ of above-mentioned EDCs were calculated for each sampling campaign and sampling station using MEC-results of both the active and passive sampling approaches (see previous section). The results of the calculated RQs are depicted in Figure 6. Lower RQs were generally observed for passive samplers in contrast to the active samples. The latter is not surprising, because this study has demonstrated that the measured concentrations for passive samplers are on average a factor of 5.3 to 31 lower than for active samples.

Among the studied EDCs, the detected concentrations for 17β-estradiol exceeded in almost all samples the proposed EQS. According to the most recent 17β-estradiol EQS dossier [66], and supported by the available chronic toxicity data (such as no observed effect concentrations, NOEC), it turns out that the most sensitive organisms to 17β-estradiol are fish [69–72] in contrast to invertebrates [73–76]. For example, exposure to 17β-estradiol can pose negative effects in the following fish species: *Onchorhynchus mykiss* ($NOEC_{reduced\ semen\ volume}$ *(35 days of exposure) = 0.5 ng* L^{-1} [71]), *Danio rerio* ($NOEC_{reduced\ egg\ survival}$ *(200 days of exposure) < 5 ng* L^{-1} [70] *and* $NOEC_{secondary\ sexual\ characteristics}$ *(21 days of exposure) = 5 ng* L^{-1} [69]) and *Oryzias latipes* ($NOEC_{reduced\ fertility\ of\ F0\ generation}$ *(59 days of exposure) = 2.9 ng* L^{-1} [72]*).*

The EQS of 17α-ethinylestradiol was not exceeded in the collected samples, except for SC3 in Zeebrugge. The potential risk of the measured 17α-ethinylestradiol concentration over the different sampling campaigns was, however, not consistently observed.

The EQS of DEHP was only exceeded in active samples (RQ = 2.8) of SC3 in Oostende. The calculated RQs of DEHP suggested mainly no risks (RQ < 1) from the detected concentration in the BPNS. The absence of risk of DEHP is somewhat surprising, as DEHP was the most abundant plasticizer detected (see previous section 3.2.) and prevailed mainly in the highest concentrations. As a result, it could be

hypothesized that no risk may be observed for the other measured di-phthalates in the BPNS either (assuming they have similar toxic potencies).

Finally, it should be noted that the RQ approach only takes the risk of individual compounds into account, while aquatic organisms in the BPNS are clearly not being exposed to single substances. It can indeed be anticipated that the detected EDCs may exert cumulative effects on aquatic organisms [77]. Furthermore, it should be noted that our study evaluated only the risks of those compounds for which EQS are available. Nevertheless, risks could also be calculated for compounds for which no EQS are available, by deriving EQS from ecotoxicological data of fresh- and marine waters. However, it should be highlighted that for only a limited number of compounds ecotoxicological data are available in ecotoxicological databases, such as the US-EPA Ecotox database.

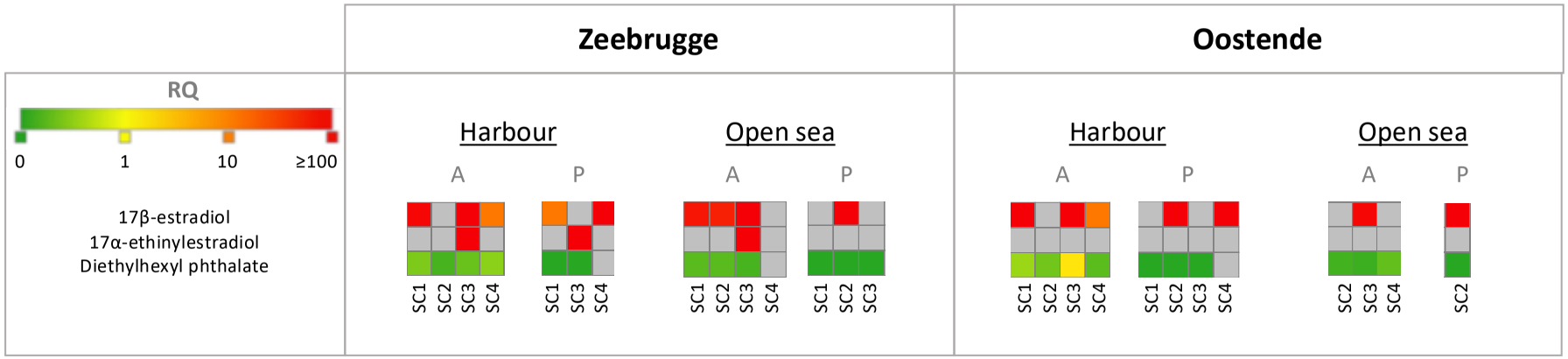


Figure 6. Calculated risk quotients (RQs) for 17β-estradiol, 17α-ethinylestradiol and diethylhexyl phthalate by using the active (A) and passive (P) sampling approach. The RQ was calculated for the different sampling campaigns (SC) between 2016 and 2018. For concentrations that were detected below the method quantification limit (MQL), the MQL was divided by two, to enable RQ calculation. The depicted RQs for the active samples are calculated based on the maximum observed concentration from samples taken during the deployment and retrieval of the passive samplers. Grey shaded cells are used for depicting the absence of calculated RQ-values, because the compound concentration was below the MDL and the EQS was below the MDL for 17β-estradiol and diethylhexyl phthalate.

4 CONCLUSIONS

A complementary sampling approach was used to assess the prevalence of 97 steroidal EDCs, plastics additives and plasticizers in the BPNS, from 2016 to 2018. Active samplers were taken by Niskin bottles, while for the passive samplers hydrophilic DVB Speedisks™ were employed. Overall, the detected concentrations for the steroidal EDCs were below 10 ng L^{-1}, while for the plastics additives and plasticizers concentrations between 10 and 1000 ng L^{-1} were observed. Similar concentrations were detected at all sampling locations, i.e. in the harbours and at open sea (fishing zone). The corticosteroids were the most abundant class of steroidal EDCs found, while dibutyl phthalate and diethylhexyl phthalate were detected at all sampling locations. Thereby, 61±11% of the total number of compounds, within the same sampling campaign, were detected by active samples, whereas the DVB Speedisks™ passive samples covered 47±17%. The concentrations measured when using passive samplers were on average a factor of 5.3 (EDCs) to 31 (plastics additives and plasticizers) lower than those in active samples.

Our study demonstrated that the detected concentrations for 17β-estradiol exceeded the EQS in almost all samples from the BPNS. For the well-known DEHP no risk was observed, although the highest concentrations were detected in contrast to other EDCs. More research regarding the ecotoxicological effects of steroidal EDCs, (alkyl)phenols and primary (mono-)phthalates is advised to enable a more complete risk assessment. Furthermore, research on the potential hazards of EDC mixtures is urgently needed in light of the results reported in this work.

REFERENCES

[1] M. Adeel, X. Song, Y. Wang, D. Francis, Y. Yang, Environmental impact of estrogens on human, animal and plant life: A critical review, Environ. Int. 99 (2017) 107–119. doi:10.1016/j.envint.2016.12.010.

[2] K. Fent, Progestins as endocrine disrupters in aquatic ecosystems: Concentrations, effects and risk assessment, Environ. Int. 84 (2015) 115–130. doi:10.1016/j.envint.2015.06.012.

[3] E.F. Orlando, A.S. Kolok, G.A. Binzcik, J.L. Gates, M.K. Horton, C.S. Lambright, et al., Endocrine-disrupting effects of cattle feedlot effluent on an aquatic sentinel species, the fathead minnow., Environ. Health Perspect. 112 (2004) 353–8. doi:10.1289/ehp.6591.

[4] G. Streck, Chemical and biological analysis of estrogenic, progestagenic and androgenic steroids in the environment, TrAC - Trends Anal. Chem. 28 (2009) 635–652. doi:10.1016/j.trac.2009.03.006.

[5] J.E. Morthorst, H. Holbech, P. Bjerregaard, Trenbolone causes irreversible masculinization of zebrafish at environmentally relevant concentrations, Aquat. Toxicol. 98 (2010) 336–343. doi:10.1016/j.aquatox.2010.03.008.

[6] K. Sone, M. Hinago, M. Itamoto, Y. Katsu, H. Watanabe, H. Urushitani, et al., Effects of an androgenic growth promoter 17β-trenbolone on masculinization of Mosquitofish (Gambusia affinis affinis), Gen. Comp. Endocrinol. 143 (2005) 151–160. doi:10.1016/j.ygcen.2005.03.007.

[7] Y.Q. Liang, G.Y. Huang, S.S. Liu, J.L. Zhao, Y.Y. Yang, X.W. Chen, et al., Long-term exposure to environmentally relevant concentrations of progesterone and norgestrel affects sex differentiation in zebrafish (Danio rerio), Aquat. Toxicol. 160 (2015) 172–179. doi:10.1016/j.aquatox.2015.01.006.

[8] M. Blázquez, S. Zanuy, M. Carrillo, F. Piferrer, Structural and functional effects of early exposure to estradiol-17β and 17α-ethynylestradiol on the gonads of the gonochoristic teleost Dicentrarchus labrax, Fish Physiol. Biochem. 18 (1998) 37–47. doi:10.1023/A:1007736110663.

[9] J.T.M. Arsenault, W.L. Fairchild, D.L. MacLatchy, L. Burridge, K. Haya, S.B. Brown, Effects of water-borne 4-nonylphenol and 17β-estradiol exposures during parr-smolt transformation on growth and plasma IGF-I of Atlantic salmon (Salmo salar L.), Aquat. Toxicol. 66 (2004) 255–265. doi:10.1016/j.aquatox.2003.09.005.

[10] A.D. Correia, S. Freitas, M. Scholze, J. Gonçalves, P. Booij, M.H. Lamoree, et al., Mixtures of estrogenic chemicals enhance vitellogenic response in sea bass, Environ. Health Perspect. 115 (2007) 115–121. doi:10.1289/ehp.9359.

[11] P.L. McNeil, C. Nebot, K.A. Sloman, Physiological and Behavioral Effects of Exposure to Environmentally Relevant Concentrations of Prednisolone during Zebrafish (Danio rerio) Embryogenesis, Environ. Sci. Technol. 50 (2016) 5294–5304. doi:10.1021/acs.est.6b00276.

[12] L. Canesi, E. Fabbri, Environmental effects of BPA: Focus on aquatic species, Dose-Response. 13 (2015) 155932581559830. doi:10.1177/1559325815598304.

[13] C. Guerranti, E. Grazioli, S. Focardi, M. Renzi, G. Perra, Levels of chemicals in two fish species from four Italian fishing areas, Mar. Pollut. Bull. 111 (2016) 449–452. doi:10.1016/j.marpolbul.2016.07.002.

[14] J. Mathieu-Denoncourt, S.J. Wallace, S.R. de Solla, V.S. Langlois, Influence of Lipophilicity on the Toxicity of Bisphenol A and Phthalates to Aquatic Organisms, Bull. Environ. Contam. Toxicol. 97 (2016) 4–10. doi:10.1007/s00128-016-1812-9.

[15] Y. Jeong, A. Schäffer, K. Smith, A comparison of equilibrium and kinetic passive sampling for the monitoring of aquatic organic contaminants in German rivers, Water Res. 145 (2018) 248–258. doi:10.1016/j.watres.2018.08.016.

[16] M. Petrovic, M. Farré, M.L. de Alda, S. Perez, C. Postigo, M. Köck, et al., Recent trends in the liquid chromatography-mass spectrometry analysis of organic contaminants in environmental samples, J. Chromatogr. A. 1217 (2010) 4004–4017.

doi:10.1016/j.chroma.2010.02.059.
[17] P. Vazquez-Roig, C. Blasco, Y. Picó, Advances in the analysis of legal and illegal drugs in the aquatic environment, TrAC - Trends Anal. Chem. 50 (2013) 65–77. doi:10.1016/j.trac.2013.04.008.
[18] D. Barceló, M. Petrovic, Challenges and achievements of LC-MS in environmental analysis: 25 years on, TrAC Trends Anal. Chem. 26 (2007) 2–11. doi:10.1016/J.TRAC.2006.11.006.
[19] F. Hernández, M. Ibáñez, R. Bade, L. Bijlsma, J. V. Sancho, Investigation of pharmaceuticals and illicit drugs in waters by liquid chromatography-high-resolution mass spectrometry, TrAC - Trends Anal. Chem. 63 (2014) 140–157. doi:10.1016/j.trac.2014.08.003.
[20] C. Ort, M.G. Lawrence, J. Reungoat, J.F. Mueller, Sampling for PPCPs in wastewater systems: Comparison of different sampling modes and optimization strategies, Environ. Sci. Technol. 44 (2010) 6289–6296. doi:10.1021/es100778d.
[21] S. Huysman, L. Van Meulebroek, F. Vanryckeghem, H. Van Langenhove, K. Demeestere, L. Vanhaecke, Development and validation of an ultra-high performance liquid chromatographic high resolution Q-Orbitrap mass spectrometric method for the simultaneous determination of steroidal endocrine disrupting compounds in aquatic matrices, Anal. Chim. Acta. 984 (2017). doi:10.1016/j.aca.2017.07.001.
[22] S. Huysman, L. Van Meulebroek, O. Janssens, F. Vanryckeghem, H. Van Langenhove, K. Demeestere, et al., Targeted quantification and untargeted screening of alkylphenols, bisphenol A and phthalates in aquatic matrices using ultra-high-performance liquid chromatography coupled to hybrid Q-Orbitrap mass spectrometry, Anal. Chim. Acta. 1049 (2019) 141–151. doi:10.1016/j.aca.2018.10.045.
[23] R. Loos, Analytical Methods for the new proposed Priority Substances of the European Water Framework Directive (WFD), JRC Tech. Rep. (2012) 71. doi:10.2788/51497.
[24] T. Schulze, M. Ahel, J. Ahlheim, S. Aït-Aïssa, F. Brion, C. Di Paolo, et al., Assessment of a novel device for onsite integrative large-volume solid phase extraction of water samples to enable a comprehensive chemical and effect-based analysis, Sci. Total Environ. 581–582 (2017) 350–358. doi:10.1016/j.scitotenv.2016.12.140.
[25] G. Poulier, S. Lissalde, A. Charriau, R. Buzier, F. Delmas, K. Gery, et al., Can POCIS be used in Water Framework Directive (2000/60/EC) monitoring networks? A study focusing on pesticides in a French agricultural watershed, Sci. Total Environ. 497–498 (2014) 282–292. doi:10.1016/J.SCITOTENV.2014.08.001.
[26] S. Liu, G.-G. Ying, R.-Q. Zhang, L.-J. Zhou, H.-J. Lai, Z.-F. Chen, Fate and occurrence of steroids in swine and dairy cattle farms with different farming scales and wastes disposal systems, Environ. Pollut. 170 (2012) 190–201. doi:10.1016/J.ENVPOL.2012.07.016.
[27] S. Liu, G.-G. Ying, L.-J. Zhou, R.-Q. Zhang, Z.-F. Chen, H.-J. Lai, Steroids in a typical swine farm and their release into the environment, Water Res. 46 (2012) 3754–3768. doi:10.1016/J.WATRES.2012.04.006.
[28] Z. Fan, S. Wu, H. Chang, J. Hu, Behaviors of Glucocorticoids, Androgens and Progestogens in a Municipal Sewage Treatment Plant: Comparison to Estrogens, Environ. Sci. Technol. 45 (2011) 2725–2733. doi:10.1021/es103429c.
[29] S. Liu, G.-G. Ying, J.-L. Zhao, F. Chen, B. Yang, L.-J. Zhou, et al., Trace analysis of 28 steroids in surface water, wastewater and sludge samples by rapid resolution liquid chromatography–electrospray ionization tandem mass spectrometry, J. Chromatogr. A. 1218 (2011) 1367–1378. doi:10.1016/j.chroma.2011.01.014.
[30] S.S. Liu, G.G. Ying, Y.S. Liu, Y.Y. Yang, L.Y. He, J. Chen, et al., Occurrence and removal of progestagens in two representative swine farms: Effectiveness of lagoon and digester treatment, Water Res. 77 (2015) 146–154. doi:10.1016/j.watres.2015.03.022.
[31] A. Garcia-Rodriguez, V. Matamoros, C. Fontas, V. Salvado, The ability of biologically based wastewater treatment systems to remove emerging organic contaminants--a review, Environ. Sci. Pollut. Res. Int. 21 (2014) 11708–11728. doi:10.1007/s11356-

013-2448-5.
[32] Y. Liu, Y. Guan, Q. Gao, N.F.Y. Tam, W. Zhu, Cellular responses, biodegradation and bioaccumulation of endocrine disrupting chemicals in marine diatom Navicula incerta, Chemosphere. 80 (2010) 592–599. doi:10.1016/j.chemosphere.2010.03.042.
[33] K.M. Lai, M.D. Scrimshaw, J.N. Lester, Prediction of the bioaccumulation factors and body burden of natural and synthetic estrogens in aquatic organisms in the river systems, Sci. Total Environ. 289 (2002) 159–168. doi:10.1016/S0048-9697(01)01036-1.
[34] E.J. Tiedeken, A. Tahar, B. McHugh, N.J. Rowan, Monitoring, sources, receptors, and control measures for three European Union watch list substances of emerging concern in receiving waters – A 20 year systematic review, Sci. Total Environ. 574 (2017) 1140–1163. doi:10.1016/j.scitotenv.2016.09.084.
[35] S. Huysman, L. Van Meulebroek, O. Janssens, F. Vanryckeghem, H. Van Langenhove, K. Demeestere, et al., Targeted quantification and untargeted screening of alkylphenols, bisphenol A and phthalates in aquatic matrices using ultra-high-performance liquid chromatography coupled to hybrid Q-Orbitrap mass spectrometry, Anal. Chim. Acta. 1049 (2019) 141–151. doi:10.1016/j.aca.2018.10.045.
[36] B.S. Halpern, S. Walbridge, K.A. Selkoe, C. V Kappel, F. Micheli, C. D'Agrosa, et al., A global map of human impact on marine ecosystems., Science. 319 (2008) 948–52. doi:10.1126/science.1149345.
[37] A.D. Tappin, G.E. Millward, The English Channel: Contamination status of its transitional and coastal waters, Mar. Pollut. Bull. 95 (2015) 529–550. doi:10.1016/j.marpolbul.2014.12.012.
[38] J.I.S. Avila, T. Kretzschmar, Simultaneous Determination of Polycyclic Aromatic Hydrocarbons, Alkylphenols, Phthalate Esters and Polychlorinated Biphenyls in Environmental Waters Based on Headspace-Solid Phase Microextraction Followed by Gas Chromatography - Tandem Mass Spectrometry, J. Environ. Anal. Chem. 04 (2017). doi:10.4172/2380-2391.1000226.
[39] A. Paluselli, V. Fauvelle, N. Schmidt, F. Galgani, S. Net, R. Sempéré, Distribution of phthalates in Marseille Bay (NW Mediterranean Sea), Sci. Total Environ. 621 (2018) 578–587. doi:10.1016/j.scitotenv.2017.11.306.
[40] J. Sánchez-Avila, J. Bonet, G. Velasco, S. Lacorte, Determination and occurrence of phthalates, alkylphenols, bisphenol A, PBDEs, PCBs and PAHs in an industrial sewage grid discharging to a Municipal Wastewater Treatment Plant, Sci. Total Environ. 407 (2009) 4157–4167. doi:10.1016/j.scitotenv.2009.03.016.
[41] L.-P. Zhang, X.-H. Wang, M.-L. Ya, Y.-L. Wu, Y.-Y. Li, Z. Zhang, Levels of endocrine disrupting compounds in South China Sea., Mar. Pollut. Bull. 85 (2014) 628–33. doi:10.1016/j.marpolbul.2013.12.040.
[42] N.H. Torres, M.M. Aguiar, L.F.R. Ferreira, J.H.P. Américo, Â.M. Machado, E.B. Cavalcanti, et al., Detection of hormones in surface and drinking water in Brazil by LC-ESI-MS/MS and ecotoxicological assessment with Daphnia magna, Environ. Monit. Assess. 187 (2015) 379. doi:10.1007/s10661-015-4626-z.
[43] B. Petrie, J. Youdan, R. Barden, B. Kasprzyk-Hordern, Multi-residue analysis of 90 emerging contaminants in liquid and solid environmental matrices by ultra-high-performance liquid chromatography tandem mass spectrometry, J. Chromatogr. A. 1431 (2016) 64–78. doi:10.1016/j.chroma.2015.12.036.
[44] P.B. Fayad, M. Prévost, S. Sauvé, On-line solid-phase extraction coupled to liquid chromatography tandem mass spectrometry optimized for the analysis of steroid hormones in urban wastewaters, Talanta. 115 (2013) 349–360. doi:10.1016/j.talanta.2013.05.038.
[45] A.L. Heffernan, K. Thompson, G. Eaglesham, S. Vijayasarathy, J.F. Mueller, P.D. Sly, et al., Rapid, automated online SPE-LC-QTRAP-MS/MS method for the simultaneous analysis of 14 phthalate metabolites and 5 bisphenol analogues in human urine, Talanta. 151 (2016) 224–233. doi:10.1016/j.talanta.2016.01.037.
[46] S.X.L. Goh, A. Duarah, L. Zhang, S.A. Snyder, H.K. Lee, Online solid phase extraction

with liquid chromatography–tandem mass spectrometry for determination of estrogens and glucocorticoids in water, J. Chromatogr. A. 1465 (2016) 9–19. doi:10.1016/J.CHROMA.2016.08.040.

[47] T. Anumol, S. a Snyder, Rapid analysis of trace organic compounds in water by automated online solid-phase extraction coupled to liquid chromatography–tandem mass spectrometry, Talanta. 132 (2015) 77–86. doi:10.1016/j.talanta.2014.08.011.

[48] A. International, ASTM D-1141-98(2013) Seawater, 2013.

[49] Royal Decree, Marine Spatial Plan for the Belgian part of the North Sea, 2014. https://www.health.belgium.be/sites/default/files/uploads/fields/fpshealth_theme_file/19094275/Summary Marine Spatial Plan.pdf (accessed January 7, 2019).

[50] E.M. Ferguson, M. Allinson, G. Allinson, S.E. Swearer, K.L. Hassell, Fluctuations in natural and synthetic estrogen concentrations in a tidal estuary in south-eastern Australia, Water Res. 47 (2013) 1604–1615. doi:10.1016/j.watres.2012.12.020.

[51] B. Vrana, I.J. Allan, R. Greenwood, G.A. Mills, E. Dominiak, K. Svensson, et al., Passive sampling techniques for monitoring pollutants in water, TrAC - Trends Anal. Chem. 24 (2005) 845–868. doi:10.1016/j.trac.2005.06.006.

[52] EC-JRC, Technical guidance document on risk assessment in support of Commission Directive 93/67/ EEC on risk assessment for new notified substances and Commission Regulation (EC) No. 1488/94 on risk assessment for existing substances. Part II. EUR 20418 EN/2, Eur. Chem. Bur. Part II (2003) 7–179. https://echa.europa.eu/documents/10162/16960216/tgdpart2_2ed_en.pdf (accessed January 7, 2019).

[53] P. Whitehouse, B. Brown, H. Wilkinson, A.B. Paya-Perez, J. Zaldivar-Comenges, K. Daginnus, et al., Common Implementation Strategy for the Water Framework Directive (2000 / 60 / EC) Guidance Document No . 27 Technical Guidance For Deriving Environmental Quality Standards, 2011. doi:10.2779/43816.

[54] M.D. Hernando, M. Mezcua, A.R. Fernández-Alba, D. Barceló, Environmental risk assessment of pharmaceutical residues in wastewater effluents, surface waters and sediments, in: Talanta, Elsevier, 2006: pp. 334–342. doi:10.1016/j.talanta.2005.09.037.

[55] P. Verlicchi, M. Al Aukidy, E. Zambello, Occurrence of pharmaceutical compounds in urban wastewater: Removal, mass load and environmental risk after a secondary treatment—A review, Sci. Total Environ. 429 (2012) 123–155. doi:10.1016/J.SCITOTENV.2012.04.028.

[56] P.J. Barnes, Anti-inflammatory Actions of Glucocorticoids: Molecular Mechanisms, Clin. Sci. 94 (1998) 557–572. doi:10.1042/cs0940557.

[57] S. Wu, A. Jia, K.D. Daniels, M. Park, S.A. Snyder, Trace analysis of corticosteroids (CSs) in environmental waters by liquid chromatography–tandem mass spectrometry, Talanta. 195 (2019) 830–840. doi:10.1016/j.talanta.2018.11.113.

[58] H. Chang, J. Hu, B. Shao, Occurrence of natural and synthetic glucocorticoids in sewage treatment plants and receiving river waters, Environ. Sci. Technol. 41 (2007) 3462–3468. doi:10.1021/es062746o.

[59] K. Schmidt-Bäumler, T. Heberer, H.J. Stan, Occurrence and distribution of organic contaminants in the aquatic system in Berlin. Part II: Substituted phenols in Berlin surface water, Acta Hydrochim. Hydrobiol. 27 (1999) 143–149. doi:10.1002/(SICI)1521-401X(199905)27:3<143::AID-AHEH143>3.0.CO;2-9.

[60] J. Sánchez-avila, J. Bonet, G. Velasco, S. Lacorte, Science of the Total Environment Determination and occurrence of phthalates , alkylphenols , bisphenol A , PBDEs , PCBs and PAHs in an industrial sewage grid discharging to a Municipal Wastewater Treatment Plant, Sci. Total Environ. 407 (2009) 4157–4167. doi:10.1016/j.scitotenv.2009.03.016.

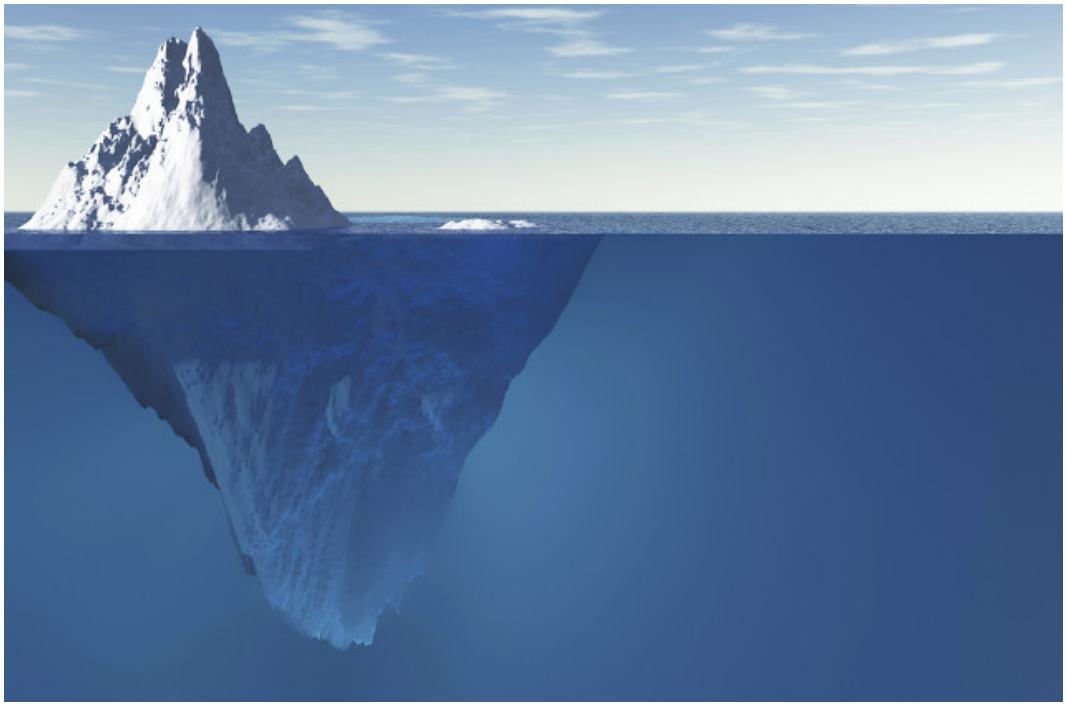
[61] Q.Y. Cai, C.H. Mo, Q.T. Wu, Q.Y. Zeng, A. Katsoyiannis, Occurrence of organic contaminants in sewage sludges from eleven wastewater treatment plants, China, Chemosphere. 68 (2007) 1751–1762. doi:10.1016/j.chemosphere.2007.03.041.

[62] P. Serôdio, J.M.F. Nogueira, Considerations on ultra-trace analysis of phthalates in drinking water, Water Res. 40 (2006) 2572–2582. doi:10.1016/j.watres.2006.05.002.

[63] X. Hu, Y. Gu, W. Huang, D. Yin, Phthalate monoesters as markers of phthalate contamination in wild marine organisms, Environ. Pollut. 218 (2016) 410–418. doi:10.1016/j.envpol.2016.07.020.
[64] A. Ramesh Kumar, P. Sivaperumal, Analytical methods for the determination of biomarkers of exposure to phthalates in human urine samples, TrAC - Trends Anal. Chem. 75 (2016) 151–161. doi:10.1016/j.trac.2015.06.008.
[65] E. Terzopoulou, D. Voutsa, Active and passive sampling for the assessment of hydrophilic organic contaminants in a river basin-ecotoxicological risk assessment, Environ. Sci. Pollut. Res. 23 (2016) 5577–5591. doi:10.1007/s11356-015-5760-4.
[66] Beta-estradiol EQS dossier, 2011. https://circabc.europa.eu/sd/a/c5356fa7-be0e-4d3b-b199-208d6e144a91/E2 EQS dossier 2011.pdf.
[67] Alpha-ethinylestradiol EQS dossier, 2011. https://circabc.europa.eu/sd/a/efb00adc-ece7-4bec-a611-3b612cf1f942/EE2 EQS dossier 2011.pdf.
[68] Priortiy Substance No 12 Diethylhexyl phthalate (DEHP), 2005. https://circabc.europa.eu/d/a/workspace/SpacesStore/337d62ba-6a8f-49ce-9c0e-591bb248e560/12_DEHP_EQS_Final Data Sheet.pdf.
[69] F. Brion, C.R. Tyler, X. Palazzi, B. Laillet, J.M. Porcher, J. Garric, et al., Impacts of 17β-estradiol, including environmentally relevant concentrations, on reproduction after exposure during embryo-larval-, juvenile- and adult-life stages in zebrafish (Danio rerio), Aquat. Toxicol. 68 (2004) 193–217. doi:10.1016/j.aquatox.2004.01.022.
[70] J.P. Nash, D.E. Kime, L.T.M. Van der Ven, P.W. Wester, F. Brion, G. Maack, et al., Long-term exposure to environmental concentrations of the pharmaceutical ethynylestradiol causes reproductive failure in fish, Environ. Health Perspect. 112 (2004) 1725–1733. doi:10.1289/ehp.7209.
[71] F. Lahnsteiner, B. Berger, M. Kletzl, T. Weismann, Effect of 17β-estradiol on gamete quality and maturation in two salmonid species, Aquat. Toxicol. 79 (2006) 124–131. doi:10.1016/j.aquatox.2006.05.011.
[72] M. Seki, H. Yokota, M. Maeda, K. Kobayashi, Fish full life-cycle testing for 17β-estradiol on medaka (Oryzias latipes), Environ. Toxicol. Chem. 24 (2005) 1259–1266. doi:10.1897/04-379R.1.
[73] N. Tatarazako, Y. Takao, K. Kishi, N. Onikura, K. Arizono, T. Iguchi, Styrene dimers and trimers affect reproduction of daphnid (Ceriodaphnia dubia), Chemosphere. 48 (2002) 597–601. doi:10.1016/S0045-6535(02)00119-4.
[74] Z. Billinghurst, A.S. Clare, T. Fileman, J. Mcevoy, J. Readman, M.H. Depledge, Inhibition of barnacle settlement by the environmental oestrogen 4-nonylphenol and the natural oestrogen 17β oestradiol, Mar. Pollut. Bull. 36 (1998) 833–839. doi:10.1016/S0025-326X(98)00074-5.
[75] M. Breitholtz, B.E. Bengtsson, Oestrogens have no hormonal effect on the development and reproduction of the harpacticoid copepod Nitocra spinipes, Mar. Pollut. Bull. 42 (2001) 879–886. doi:10.1016/S0025-326X(01)00046-7.
[76] T.H. Hutchinson, N.A. Pounds, M. Hampel, T.D. Williams, Impact of natural and synthetic steroids on the survival, development and reproduction of marine copepods (Tisbe battagliai), in: Sci. Total Environ., Elsevier, 1999: pp. 167–179. doi:10.1016/S0048-9697(99)00223-5.
[77] S. Firpo, Mixture and single-compound toxicity using Daphnia magna-Comparisons with estimates of concentration addition and independent action, n.d. http://stud.epsilon.slu.se (accessed February 6, 2019).

V

General discussion and future perspectives



1 RESEARCH POSITION AND RELEVANCE OF THIS THESIS

This doctoral thesis is embedded in the NewSTHEPS project (www.newstheps.be), focusing on New Strategies for monitoring and risk assessment of Hazardous Chemicals in the marine Environment with Passive Samplers. NewSTHEPS intends to establish an innovative and integrated approach to assess the risks posed by mixtures of micropollutants in the Belgian coastal zone, thereby addressing the following specific aims:

1) To develop a comprehensive multi-method approach to monitor (target analysis) and screen (untargeted analysis) a broad range of waterborne pollutants (organic compounds and metals);
2) To apply a unique combination of field and laboratory ecotoxicological and chemical techniques to establish the environmental risk of realistic mixtures of contaminants;
3) To develop and evaluate a framework and toolbox for monitoring the chemical anthropogenic pressures on coastal ecosystems.

The objectives of this doctoral thesis were mainly related to the green section in Figure 1, including the development of new analytical multi-class methods for the detection/quantification of micropollutants in the BPNS and the use and calibration of passive samplers. Because EDCs are an important class of micropollutants posing a potential threat but lacking prior assessment for the marine environment, this doctoral thesis mainly focused on a number of important EDC groups. Indeed, the prevalence of steroidal EDCs and plastics additives and plasticizers has recently gained attention as a result of their frequent use in daily life and endocrine disrupting properties towards marine organisms [1–6].

To study and evaluate the fate, effects and risks posed by these EDCs in aquatic ecosystems, information regarding their presence in the marine environment is urgently needed. Therefore, the main goal of this doctoral thesis was to fill up the important gap in knowledge on integrated monitoring approaches of steroidal EDCs, plastics additives and plasticizers in the BPNS. The

latter implicates the need for new and reliable analytical methods for the measurement of multiple EDC classes in aqueous samples.

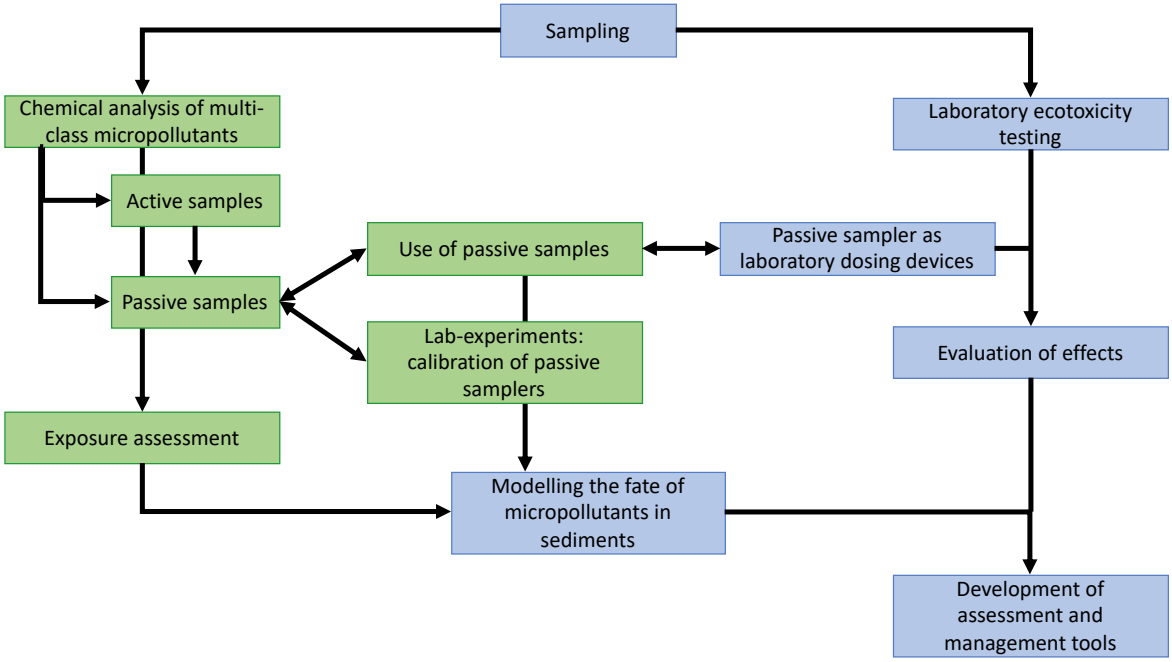


Figure 1. Schematic overview of the objectives of the NewSTHEPS project (BR/143/A2/NEWSTHEPS).

2 MAIN RESEARCH FINDINGS AND SCIENTIFIC CONTRIBUTIONS OF THIS WORK

The general aim of this doctoral thesis was to study the occurrence of EDCs in the marine BPNS environment using both active and passive sampling approaches followed by HRMS-based instrumentel analysis. The accomplishment of this objective has been extensively described in chapters II, III and IV. The main findings are summarized in Figure 2 and will be further discussed throughout this chapter (V).

2.1 Active sampling

The most frequently reported steroidal EDCs, plastics additives and plasticizers are the oestrogens and di-phthalates, whereas data for androgens, progestagens, corticosteroids and mono-phthalates are more scarce [7–62].

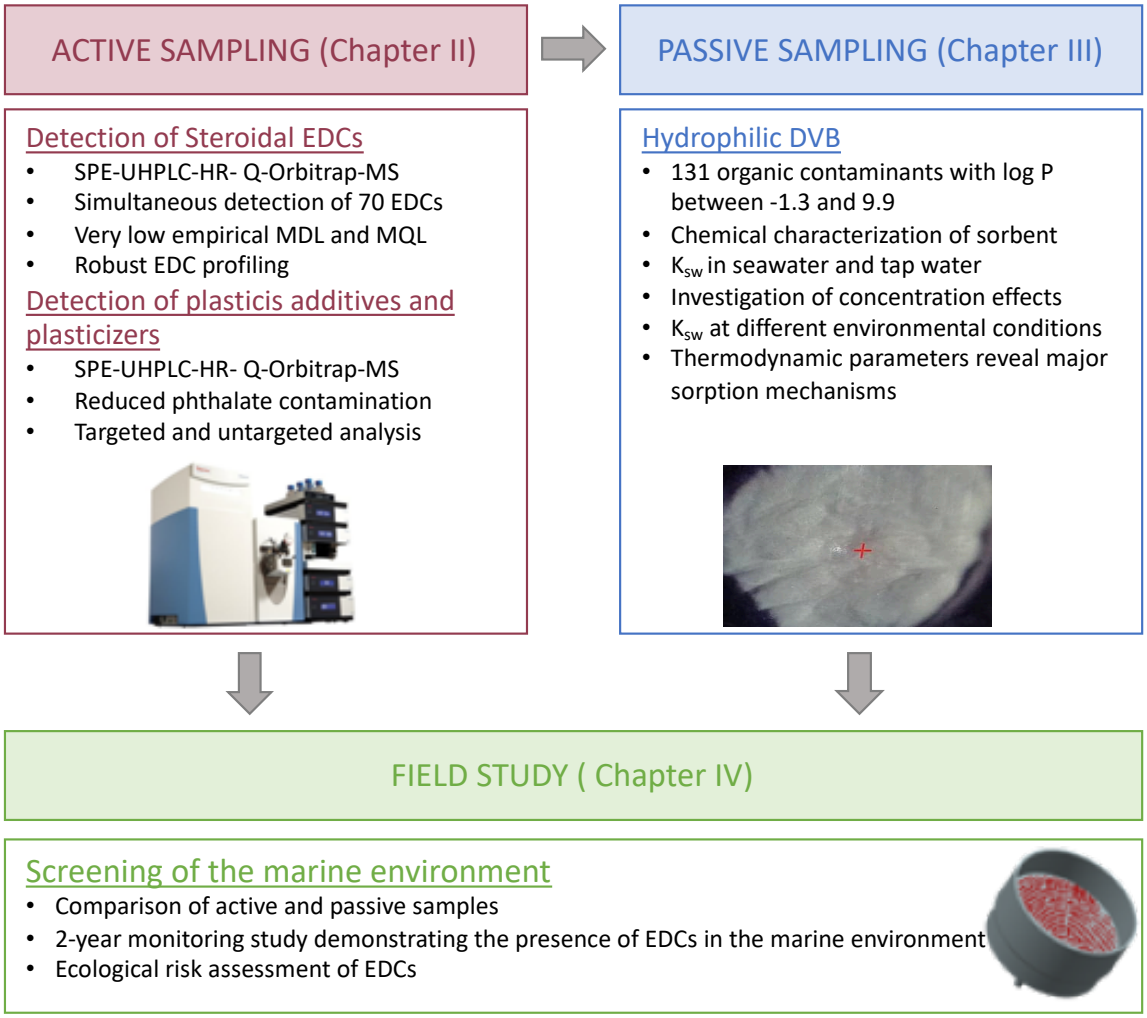


Figure 2. Schematic overview of the main research findings of this PhD study.

Because of differences in physico-chemical properties and chemical backbone between the steroidal EDCs and the plastics additives and plasticizers, two different analytical methods making use of solid-phase extraction and UHPLC-HRMS were developed in this work.

2.1.1 UHPLC-HR-Q-Orbitrap-MS methods development and validation for target EDC analysis

2.1.1.1 Trace quantification of steroidal EDCs

During this work, a UHPLC-HR-Q-Orbitrap-MS method was developed for the simultaneous quantification of 70 steroidal EDCs in two aquatic matrices, i.e. sea and fresh water. Prior to the quantification of the analytes, there was a need to separate the isomers and mass analogues, as to achieve a reliable quantification. To achieve EDC separation, UHPLC was

selected as main technique for enabling superior resolution, chromatographic peak shape and baseline separation of analytes. In comparison to classical HPLC, UHPLC has demonstrated to provide a higher resolution (5-fold), speed (10-fold), sensitivity (analyte specific) and a reduced solvent consumption (5-fold) [63,64]. When comparing the UHPLC optimization step in this work to similar studies from literature, it is reasonable to conclude that our methods offer a higher throughput (4-fold) for the simultaneous separation of EDCs [17,65,66]. To enable a reliable and accurate quantification of the separated EDCs, the ionization and other relevant mass spectrometric parameters were optimized on spiked seawater extracts. This resulted in high mass accuracies (mass deviation < 3 ppm) at MQL levels. The latter was deemed highly important to increase the confidence in identification, as in the marine environment it was expected that low ng L^{-1} concentrations would be detected. Next to the UHPLC-HR-Q-Orbitrap-MS method, that was fully optimized to measure residual concentrations in the environment, the initial sampling and extraction procedure are also known to play an equivalent or even more important role in the ability of detecting residues in the marine environment. To tackle the latter, large volume of water samples are usually needed [67], followed by an SPE procedure for up-concentrating analytes [68]. In this work, the SPE procedure was optimised in a very cost-effective manner by using a three-step workflow, including fractional factorial resolution IV (screening), simplex lattice and response surface methodological designs.

Finally, the performance of the optimised SPE-procedure coupled to the instrumental UHPLC-HR-Q-Orbitrap-MS method was evaluated by an extensive validation, relying on strict criteria, i.e. CD 2002/657/EC (food safety) [69], the Eurachem guidelines [70] and review articles [71,72]. Moreover, this validation was performed at environmentally relevant concentrations, resulting in very low quantification limits (all below 5 ng L^{-1}) and excellent performance characteristics [73]. Consequently, the applicability was demonstrated by measuring steroidal EDCS in 28 sea water samples, during which it was shown that many (n=43) steroidal EDCs were ubiquitously present at low concentrations (0.28 – 39 ng L^{-1}). This observation confirmed the existing need for an analytical methodology that enables the detection of a broad range of

steroidal EDCs at the obtained MQL-levels for environmental purposes. Ultimately, it could be concluded that the developed and validated method is fit-for-purpose, which was fundamental for the calibration and use of passive sampling-based devices. This was further investigated in chapters III and IV.

2.1.1.2 Detection of plastics additives and plasticizers

GC generally hampers the analysis of higher molecular phthalates, as a result of their intermediate volatility, while this is not the case for LC. As it was the purpose to quantify a broad range of plastics additives and plasticizers – not limited to the volatile phthalates – LC was the chromatographic platform of choice. Nevertheless, the use of UHPLC for separating plastic additives and plasticizers is challenging from an analytical perspective. First, instability of retention times was observed for the alkylphenols when formic acid was added to the mobile phase. Therefore, ammonium hydroxide was selected as a mobile phase additive, bearing the additional benefit of an enhanced ionization rate for the alkylphenols. Second, interfering background peaks of diethyl hexyl and dinonyl phthalate were observed in almost every analytical run. To solve this, a trap column was placed between the UHPLC pump and the injection valve for retarding any potential plastics additive and phthalate contaminations. Third, transesterification of di-phthalate to primary monomethyl phthalate was observed when methanol was used as a mobile phase. For controlling the degree of transesterification of target phthalates and potentially unknown (untargeted) phthalates, acetonitrile was selected as the mobile phase. Subsequent to the LC optimization, the ionization and mass spectrometric settings were optimised and developed in a similar manner as described under 2.1.1.1. A similar validation strategy was also applied, as only diethyl phthalate was covered by an EQS level in CD 2009/90/EC. Validation likewise provided superior analytical performance for all target plasticizers and plastics additives. Consequently, this method was applied on 24 seawater samples, clearly demonstrating the presence of mono-phthalates and confirming the need for also including transformation products in the analysis. This instrumental method formed one of the fundaments for calibrating the passive sampler-based devices, as reported in chapters III and IV.

2.1.2 Suspect and untargeted analysis using the developed UHPLC-HR-Q-Orbitrap-MS methods

The developed and validated multi-residue methods targeting steroidal EDCs, plastics additives and plasticizers, described in chapter II, made use of high-end UHPLC-HR-Q-Orbitrap-MS. This MS technology simultaneously enables to perform suspect and untargeted analysis, which can both contribute to further improving water quality monitoring. In the context of untargeted analysis, most studies use analytical columns with a length of at least 100 mm and maximal particle diameter of 2.5 µm, resulting in an excellent chromatographic resolution (narrow peaks) [74]. Also in this work, the use of small particle sizes and a sufficiently high length of the UHPLC column both resulted in a reduced plate height of the column [75] and a high peak capacity for the separation of the analytes under study. The strength of UHPLC was also clearly demonstrated for the steroidal EDCs, as our developed method enabled the separation of chiral and mass analogues [73]. In response to the narrow LC-peaks obtained in this study, UHPLC was hyphenated to high resolution mass spectrometry, i.e. a Q-Orbitrap as is common for untargeted analyses. Indeed, HRMS makes it possible to detect a virtually unlimited number of compounds in a single run without preselection. Several studies have already demonstrated that thousands of substances may be detected in the aquatic environment, with target quantification only covering the tip of the iceberg [36,76,77]. Furthermore, HRMS provides reliable detection and quantification of the separated analytes, and this with high mass accuracies. For the examined target EDCs (chapter II), high mass accuracies (< 3 ppm) were also observed [73,78].

The developed and validated UHPLC-HR-Q-Oribtrap-MS methods, described in chapter II, meet all the above-mentioned instrumental requirements for performing suspect and untargeted analyses [73,79]. Suspect and untargeted analysis, which are mostly executed in retrospect, generally rely on accurate mass, retention time and spectral libraries [80–83].

However, relying merely on these data brings about a certain degree of uncertainty related to the component identity, which can be minimized by fragmenting ‘suspect’ or ‘unknown’

compounds. For the plastics additives and plasticizers, an analytical strategy was proposed in this work for the tentative identification by using in-house determined fragments (incorporated in Python) of compounds, using Tier 3 (according to the Chemical Analysis Working Group & Metabolomics Standards Initiative) [84].

2.2 Passive sampling using hydrophilic divinylbenzene

Passive sampling techniques have been initially developed as an alternative for overcoming the challenges of active sampling, as extensively described in section 3.1 of chapter I. From literature, it has become clear that each commercially available polymeric passive sampler only captures a limited range of analytes [85–92]. This is governed by the sampler's polarity and chemical moieties of the sorbent surface, but also by the physico-chemical properties of the analytes. The incorporation of the Oasis HLB® co-polymer in different POCIS designs is one of the most popular passive sampler devices, as it permits the accumulation of very polar to moderately non-polar compounds (log P ranging from -1.6 to 5) [91]. Nevertheless, the simultaneous detection of polar and non-polar compounds by one passive sampler seems to offer a hurdle so far. This stresses the need for investigating novel passive sampler devices that may allow to simultaneously capture both polar and non-polar organic contaminants from aquatic matrices.

In this work, we demonstrated that higher extraction efficiencies were obtained for non-polar compounds (log P > 4) with hydrophilic DVB (polymeric sorbent in Speedisks) as compared to Oasis® HLB when using both sorbents during SPE clean-up of grab samples [73]. On top of that, active sampling of steroidal EDCs demonstrated that a broad polarity range was captured by hydrophilic DVB (log P ranging from 0.7 to 9). Therefore, we proposed to use hydrophilic DVB as a novel sorbent for passive sampling approaches. A supplementary benefit relied on the fact that hydrophilic DVB was incorporated in a robust configuration, i.e. Bakerbond Speedisks, which can be directly used as passive sampler devices in the environment. To evaluate the potential of hydrophilic DVB for capturing compounds with a broad polarity range, we extended – within the NewSTHEPS project – the scope of target

compounds towards a more extensive range of emerging contaminants (log P ranging from -1.3 to 9.85), i.e. personal care products (n=5), pharmaceuticals (n=32), pesticides (n=25), steroidal EDCs (n=50), (alkyl)phenols (n=4), and phthalates (n=15). The analysis and evaluation of the uptake of personal care products, pharmaceuticals and pesticides was executed by Vanryckegem and colleagues at the EnVOC Research Group. Combining the experimentally measured equilibrium partitioning coefficients (K_{sw}) of 131 organic contaminants with the functional characteristics (determined via FTIR and NIR) of the hydrophilic DVB sorbent, indicated its superiority for capturing organic compounds within the aforementioned broad polarity range. In parallel to this study, Vanryckegem et al. demonstrated that the Speedisk itself can be applied as an equilibrium passive sampler for the same set of emerging organic compounds. The determined K_{sw} values were used in chapter IV to convert the accumulated mass (ng) of organic compounds on the Speediks to analyte water concentrations (ng L^{-1}).

2.3 Field study – Screening of the marine environment

In chapter IV, the two studied sampling approaches, i.e. active and passive sampling, were extensively evaluated by application to the marine environment. This approach was excecuted in the Belgian Part of the North Sea, for assessing the presence of 70 steroidal EDCs, and 27 plastics additives and plasticizers.

The analysis of the active and passive samples resulted in detected concentrations for the steroidal EDCs below 10 ng L^{-1}, while for the plastics additives and plasticizers concentrations between 10 and 1000 ng L^{-1} were observed. Comparing our results to concentrations reported for other aquatic environments (see Figure 3 of the introduction), it can be concluded that similar ranges were detected in the marine environment as previously reported for surface, ground and drinking water. Deeper investigation of the EDC sub-classes showed that the corticosteroids were the most abundant class of steroidal EDCs, while dibutyl phthalate and diethylhexyl phthalate were found at all sampling locations in BPNS, from 2016 to 2018.

Comparing the complementarity of active and passive sampling showed that 61±11% of the total number of compounds were detected by active samples, whereas the DVB Speedisks™ passive samplers covered 47±17%. The overlapping coverage between active and passive samples was 31±14%. The concentrations that were detected by using passive samplers were on an average a factor of 5.3 (steroidal EDCs) to 31 (plastics additives and plasticizers) lower than with active samples. Futhermore, passive samplers can act as “early-warning tool” to detect trends in chemical contaminants [93]. Concentrations that are detected outside the upper and lower central limits of previous sampling campaigns, can be used to trigger further monitoring using the conventional active (grab) sampling. Consequently, passive sampling can be recommended, as a complementary tool to improve water quality, in the European Commission Guidance document [94] and in Directive 2013/39/EU [95] for the chemical monitoring of surface waters. Passive samplers allow the measurement of the dissolved phase concentrations of a contaminant [93]. However, for polar to moderately polar organic compounds (with log P < 5), the concentration in the water column is not dominated by the fraction adsorbed to colloids and particles in water, while this is the case for the more non-polar compounds [93]. As such, assessing the polar to moderately polar organic compounds by using passive sampling in compliance with the EQS of the European Water Framework Directive (WFD), can be questioned. Therefore, more research is needed to evaluate how reliable the hydrophilic DVB Speedisk is under the WFD for assessing the more polar compounds. Finally, the risk quotient approach showed that 17β-estradiol exceeded the EQS (RQ > 1) for marine waters, while DEHP didn’t pose a risk (RQ < 1).

3 FUTURE RESEARCH RECOMMENDATIONS

3.1 Untargeted analysis

3.1.1 Structure elucidation

A crucial part in HRMS-based untargeted analysis as a novel means to optimize environmental monitoring, is the identification of unknowns. In this work, we proposed a new strategy for plastics additives and plasticizers identification, relying on class-specific fragments. An equivalent analytical strategy could be pursued for the steroidal EDCs. The feasibility of this strategy is deemed high, as different studies have demonstrated a correlation between the chemical structure and the obtained product ions for analogues of specific steroids [96–98]. Indeed, Pozo et al. (2008 and 2009) already reported that the observed fragmentation patterns of steroidal EDCs can be considered for structural elucidation [96,98]. In addition to the mass spectrometric part, a recent study of Randazzo et al. (2016) successfully proposed a tool for predicting the retention time of 91 steroids in reversed-phase liquid chromatography in association with experimental MS information. Retention time predictions were based on VolSurf+3D molecular descriptors in combination with novel stereochemical description of the gonane skeleton of steroidal EDCs (see Figure 3). The proposed tool showed a predictive ability (Q^2) of 0.92 with and average error of 4.4% [99]. Combining characteristic fragmentation patterns and prediction of retention times would allow to better unveil the identity of ‘suspect’ and ‘unknown’ compounds during retrospective analysis.

3.1.2 Automatized analysis and processing

Within this doctoral thesis, the fragmentation of plastics additives and plasticizers was executed by using full scan MS in combination with parallel reaction monitoring (PRM) events. The latter uses an inclusion list of the parent ions (with their accurate masses and retention times), measured in full-scan, for fragmenting ‘unknown’ compounds. This was followed by a pre-processing step in Python, which allowed to assign neutral losses and characteristic

fragments of the target list to the untargeted data. A more automatized fashion would be combining full scan events with data-dependent fragmentation data. Combining those two events would bring about the automatic fragmentation of the highest observed peaks at specific retention times and accurate masses (without the use of an inclusion list). Using this approach, the data evaluation and annotation could then directly be executed by using our in-house written

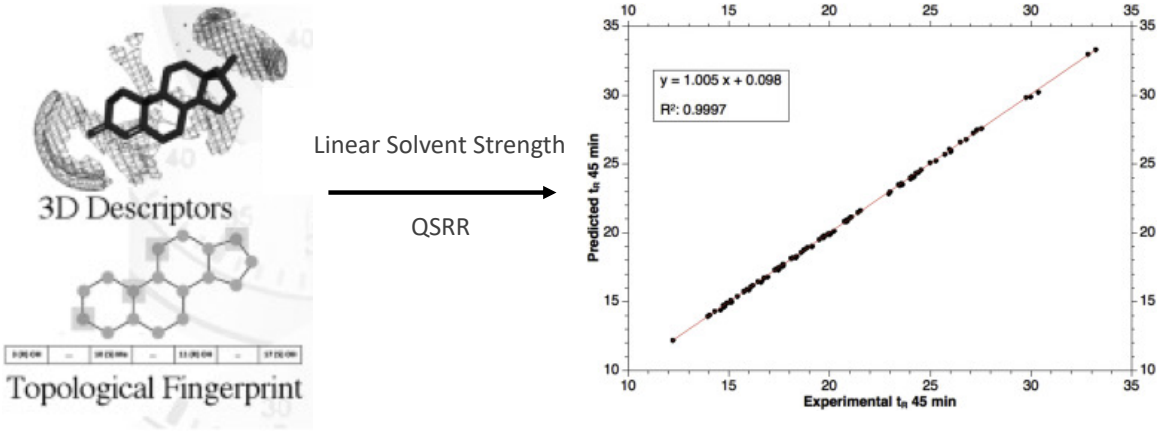


Figure 3. Schematic overview depicting the predicted retention times versus the experimental retention time (credits belong to Randazzo et al. 2016).

Python code or other freely available annotation software programs (such as MAGMA) [100]. This would provide information more rapidly than with PRM on whether or not "suspects" or "unknowns" would contain an EDC backbone.

Furthermore, further data processing could comprise peak peaking by using molecular feature algorithms, such as Compound Discover ™, Sieve, MZmine and Bioconductor (xcms) (R-package). Thereby, it should be highlighted that only Bioconductor allows to be integrated in a single pipeline together with the above-mentioned backbone-based structural elucidation. These algorithms cluster all related MS signals and report them as compounds compiled in a large list for further evaluation. Relevant peaks, that are not present in procedural blanks or may be associated with existing targets, are selected based on isotopic pattern and intensities [101]. For the selected peaks, the most plausible molecular formula can be assigned to by using the Seven Golden Rules [102] and GenForm [103]. MS/MS spectral interpretation includes the use of on-line databases (MassBank library) and fragmentation platforms (MS

Fragmenter, Mass Frontier and MetFrag). To assess the plausibility of candidates, the QSRR retention time prediction model can be applied [104]. To conclude, the above-mentioned proposed strategy can be embedded in a wide-scope screening approach aiming at the detection and identification of unknown micropollutants and their transformation products [105].

3.2 Partitioning of organic compounds between water and hydrophilic DVB

3.2.1 Further investigation of unassessed effects

The sorption of emerging organic contaminants occurring in aquatic matrices onto hydrophilic DVB has shown to be successful, as described in chapter III. Thereby, concentration effects were studied by increasing the analyte concentrations in mixture experiments. Furthermore, concentrations of mixtures that were tested, within the same batch experiment, were equal-molar concentrations. Nevertheless, in the environment, contaminants often exist in mixtures with other chemicals, as was observed in chapter IV. As a consequence, competition between compounds with different concentrations can occur, and affect the K_{sw}. Thereby, it should be highlighted that sorption behaviour in a single-solute and mixture-solute system can also differ substantially [106–108]. Compounds that are structurally related can display stronger competitive effects [109–112]. Moreover, Xing and Pignatello reported that the existence of competitive sorption between organic pollutants and aromatic acids - also called naturally occurring compounds - may cause an increasing mobility and bioavailability of anthropogenic organic contaminants [113]. For example, the study of Wang et al. (2009) showed that humic acids influence the sorption beviour, which was dominated by the molecular size and hydrophobicity of the organic pollutants. Another study by Lerman et al. (2013) observed that dissolved organic matter can significantly affect the sorptive behaviour of polar organic pollutants [114].

Next to the to above-mentioned unassessed environmental effects, the design of the passive sampler should be further examined as well. Future research shoud investigate the uptake behaviour of EDCs onto hydrophilic DVB that is enclosed in different designs.

3.2.2 Stability of emerging organic compounds on Speedisks

In this study, the stability of EDCs on Speedisks was not investigated. The sequestration of analytes onto a solid-phase sorbent prior to freezing can, however, be considered as an effective storage and archival option [115]. Indeed, two recent studies reported that pharmaceuticals, pesticides and other polar organic compounds are stable on POCIS during a storage period of 20 months and even 6 years [115,116]. Therefore, POCIS can serve as a viable archival tool for monitoring programs interested in exploring contaminant discovery on original samples at a later date. Furthermore, storage on solid-phase sorbents represents also a logistically feasible method, as opposed to the storage of large water-volume samples obtained following active sampling.

Successful sequestration of the emerging organic compounds studied in this work on Speedisks can be anticipated, when comparing the sorbent and design of POCIS to that of Speedisks. Moreover, sorbent characterisation showed that hydrophilic DVB and Oasis HLB comprise different moieties, but at the same time possess a similar spectral profile. The POCIS and Speedisk configurations both envelop a robust casing that encloses the sorbent between membranes. As a consequence, no physical damage from the freeze/thaw process will occur. Water content in the sampler during the freeze/thaw process can result in potential hydrolysis and physical damage (sorbent loss during later extraction), which was observed for the o-DGT passive sampler [117,118].

3.3 Field study

3.3.1 Practical considerations

To the best of the authors knowledge, no standard operating procedure (SOP) is available at present for passive samplers capturing polar compounds. In order to enhance transparent, comparable and reliable results, it would be a great merit to the scientific field if a SOP would be available. This SOP should encompass sampler deployment, retrieval/recovery, sampler storage, cleaning, instrumental analysis, extraction and data processing for specific classes (according to polarity). Furthermore, specific guidelines on evaluating the performance of

passive samplers, such as mass balances and linearity, would also be welcomed by the scientific community [93]. In particular, a guideline that defines a common set of metadata and calibration conditions (temperature, water flow, type of exposure system, type of water) combined with the obtained sampler calibration parameters would come in handy. This information is also required for the assessment of the possible relationship between the observed variability, available calibration data and the exposure conditions used during calibration [119]. The latter would especially be useful for new passive samplers that are introduced or variants on currently used passive samplers.

3.3.2 Water-based epidemiology

Water-based epidemiology is a multi-disciplinary domain, as it combines analytical chemistry with environmental toxicology, biology, pharmacology, public health, forensics, and so on [105]. Most of the studies so far focused on how to calculate drug consumption from wastewater analysis [120–122]. Nevertheless, only a few water-based epidemiological studies explored the association between the consumption of specific chemicals by a population, measured concentrations of metabolites in wastewater, and its health impact [123]. Other researchers used the presence of (urine-)biomarkers in wastewater to estimate the human population size during epidemiological studies [124,125]. A similar approach could be followed in the marine environment for assessing the exposure of organic contaminants to aquatic organisms and detecting unique biomarkers. The latter could be executed by performing retrospective analysis on the same samples as in chapter IV.

Another interesting track could be the calculation of product/parent ratios of organic compounds [126]. Generally, in human excretion, fixed product/parent ratios of organic compounds have been observed. Calculating and comparing the product/parent ratios of organic compounds (such as desmethyltramadol) in different aquatic environments could provide valuable information on the characteristics of the aquatic environment and any on-going processes [126]. A stable ratio would allow predicting concentrations of transformation products from those of their parents. Nevertheless, unstable ratios can be an indicator for on-

going processes (such as degradation). For example, effluent of wastewater treatment showed higher product/parent ratios of venlafaxine than human excrements, indicating that the parent compounds are more vulnerable for degradation in these WWTPs [126].

3.3.3 Passive samplers versus biota

Replacement of chemical monitoring in biota by passive samplers can be envisaged as passive samplers reflect the contaminant levels to which biota are exposed in their natural environment. Indeed, a long-term observation of passive samplers versus mussels in marine waters (Netherlands), demonstrated that similar contaminant trends in time and place/space could be observed [127]. Moreover, PDMS was used for the investigation of lipids, chlorinated biphenyls and PAHs [127]. Nevertheless, the uptake of contaminants by aquatic organisms has some drawbacks, as this is mostly dependent on the aquatic species (geographical dependence), growth stage of organisms, and variation in metabolism of aquatic organisms [128]. As a consequence, comparing the bioavailable concentration in biota on different locations could result in a discrepancy with respect to the accumulated emerging organic compounds. Therefore, the use of passive samplers could comprise a better and more reliable comparison of measured contaminants [129]. On top of that, it should be noted that passive samplers offer a more ecological and ethical solution for providing information on the contamination status of aquatic environments. Furthermore, the analysis of biota is very cost-intensive and requires extensive clean-up steps to obtain sufficient selectivity towards the target compounds [130].

3.3.4 Risk assessment of unassessed EDCs

The risk assessment of organic contaminants in the marine environment suffers from a number of limitations. First, as already highlighted in chapter IV, ecotoxicity data for marine organisms are limited. Secondly, the potential effects of organic contaminant mixtures, especially at low concentrations, is very arduous to assess and as such hardly examined. In the environment, aquatic organisms are not exposed to a single compound, but to a mixture

of organic contaminants [131]. Thirdly, only few studies take into account the presence of transformation products [132,133].

The above-mentioned issues can be addressed by using Speedisks, that were deployed in the environment, as passive dosing devices in lab toxicity tests. Moreover, the use of hydrophilic DVB Speedisks as passive dosers could provide a direct estimation of the potential toxicity to aquatic organisms. Indeed, passive samplers can be flexibly applied for testing the soluble fraction of contaminants, for performing concentration-response experiments, and for in depth investigating of the toxicity of chemical mixtures [134]. In contrast to classical bio-testing assessments, no cofounding effects of co-solvents on aquatic organisms will occur [135,136] and/or result in compound losses [137]. As a consequence, it is also expected that passive dosing will break through as a promising and widely applicable technique, as it enables the toxicity assessment of a huge number of organic contaminants in a cost-effective manner.

REFERENCES

[1] V. Christen, S. Hickmann, B. Rechenberg, K. Fent, Highly active human pharmaceuticals in aquatic systems: A concept for their identification based on their mode of action, Aquat. Toxicol. 96 (2010) 167–181. doi:10.1016/j.aquatox.2009.11.021.

[2] C.R. Tyler, S. Jobling, Roach, Sex, and Gender-Bending Chemicals: The Feminization of Wild Fish in English Rivers, Bioscience. 58 (2008) 1051. doi:10.1641/B581108.

[3] K. Fent, Progestins as endocrine disrupters in aquatic ecosystems: Concentrations, effects and risk assessment, Environ. Int. 84 (2015) 115–130. doi:10.1016/j.envint.2015.06.012.

[4] D.J. Caldwell, F. Mastrocco, T.H. Hutchinson, R. Länge, D. Heijerick, C. Janssen, et al., Derivation of an aquatic predicted no-effect concentration for the synthetic hormone, 17α-ethinyl estradiol, Environ. Sci. Technol. 42 (2008) 7046–7054. doi:10.1021/es800633q.

[5] J. Fick, R.H. Lindberg, J. Parkkonen, B. Arvidsson, M. Tysklind, D.G. Joakim Larsson, Therapeutic levels of levonorgestrel detected in blood plasma of fish: results from screening rainbow trout exposed to treated sewage effluents, Environ. Sci. Technol. 44 (2010) 2661–2666. doi:10.1021/es903440m.

[6] J. Zeilinger, T. Steger-Hartmann, E. Maser, S. Goller, R. Vonk, R. L??nge, Effects of synthetic gestagens on fish reproduction, Environ. Toxicol. Chem. 28 (2009) 2663–2670. doi:10.1897/08-485.1.

[7] S. Liu, G.-G. Ying, R.-Q. Zhang, L.-J. Zhou, H.-J. Lai, Z.-F. Chen, Fate and occurrence of steroids in swine and dairy cattle farms with different farming scales and wastes disposal systems, Environ. Pollut. 170 (2012) 190–201. doi:10.1016/J.ENVPOL.2012.07.016.

[8] S. Liu, G.-G. Ying, L.-J. Zhou, R.-Q. Zhang, Z.-F. Chen, H.-J. Lai, Steroids in a typical swine farm and their release into the environment, Water Res. 46 (2012) 3754–3768. doi:10.1016/J.WATRES.2012.04.006.

[9] L. Viglino, K. Aboulfadl, M. Prévost, S. Sauvé, Analysis of natural and synthetic estrogenic endocrine disruptors in environmental waters using online preconcentration coupled with LC-APPI-MS/MS, Talanta. 76 (2008) 1088–1096. doi:10.1016/j.talanta.2008.05.008.

[10] S. Aguayo, M.J. Muñoz, A. de la Torre, J. Roset, E. de la Peña, M. Carballo, Identification of organic compounds and ecotoxicological assessment of sewage treatment plants (STP) effluents, Sci. Total Environ. 328 (2004) 69–81. doi:10.1016/J.SCITOTENV.2004.02.013.

[11] J. Martín, D. Camacho-Muñoz, J.L. Santos, I. Aparicio, E. Alonso, Occurrence of pharmaceutical compounds in wastewater and sludge from wastewater treatment plants: Removal and ecotoxicological impact of wastewater discharges and sludge disposal, J. Hazard. Mater. 239–240 (2012) 40–47. doi:10.1016/J.JHAZMAT.2012.04.068.

[12] S. Zorita, L. Mårtensson, L. Mathiasson, Occurrence and removal of pharmaceuticals in a municipal sewage treatment system in the south of Sweden, Sci. Total Environ. 407 (2009) 2760–2770. doi:10.1016/j.scitotenv.2008.12.030.

[13] P. Hohenblum, O. Gans, W. Moche, S. Scharf, G. Lorbeer, Monitoring of selected estrogenic hormones and industrial chemicals in groundwaters and surface waters in Austria, Sci. Total Environ. 333 (2004) 185–193. doi:10.1016/j.scitotenv.2004.05.009.

[14] N.H. Torres, M.M. Aguiar, L.F.R. Ferreira, J.H.P. Américo, Â.M. Machado, E.B. Cavalcanti, et al., Detection of hormones in surface and drinking water in Brazil by LC-ESI-MS/MS and ecotoxicological assessment with Daphnia magna, Environ. Monit. Assess. 187 (2015) 379. doi:10.1007/s10661-015-4626-z.

[15] S. Rocha, V.F. Domingues, C. Pinho, V.C. Fernandes, C. Delerue-Matos, P. Gameiro, et al., Occurrence of bisphenol A, estrone, 17β-estradiol and 17α-ethinylestradiol in Portuguese rivers, Bull. Environ. Contam. Toxicol. 90 (2013) 73–78. doi:10.1007/s00128-012-0887-1.

[16] S. Rodriguez-Mozaz, M.J. López De Alda, D. Barceló, Monitoring of estrogens, pesticides and bisphenol A in natural waters and drinking water treatment plants by solid-phase extraction-liquid chromatography-mass spectrometry, in: J. Chromatogr. A, Elsevier, 2004: pp. 85–92.

doi:10.1016/j.chroma.2004.06.040.

[17] B. Petrie, J. Youdan, R. Barden, B. Kasprzyk-Hordern, Multi-residue analysis of 90 emerging contaminants in liquid and solid environmental matrices by ultra-high-performance liquid chromatography tandem mass spectrometry, J. Chromatogr. A. 1431 (2016) 64–78. doi:10.1016/j.chroma.2015.12.036.

[18] B. Pauwels, H. Noppe, H. De Brabander, W. Verstraete, Comparison of Steroid Hormone Concentrations in Domestic and Hospital Wastewater Treatment Plants, J. Environ. Eng. 134 (2008) 933–936. doi:10.1061/(ASCE)0733-9372(2008)134:11(933).

[19] S. Liu, G.G. Ying, J.L. Zhao, F. Chen, B. Yang, L.J. Zhou, et al., Trace analysis of 28 steroids in surface water, wastewater and sludge samples by rapid resolution liquid chromatography-electrospray ionization tandem mass spectrometry, J. Chromatogr. A. 1218 (2011) 1367–1378. doi:10.1016/j.chroma.2011.01.014.

[20] S.X.L. Goh, A. Duarah, L. Zhang, S.A. Snyder, H.K. Lee, Online solid phase extraction with liquid chromatography–tandem mass spectrometry for determination of estrogens and glucocorticoids in water, J. Chromatogr. A. 1465 (2016) 9–19. doi:10.1016/J.CHROMA.2016.08.040.

[21] M. Pedrouzo, F. Borrull, E. Pocurull, R.M. Marcé, Estrogens and their conjugates: Determination in water samples by solid-phase extraction and liquid chromatography–tandem mass spectrometry, Talanta. 78 (2009) 1327–1331. doi:10.1016/J.TALANTA.2009.02.005.

[22] M. Pedrouzo, F. Borrull, E. Pocurull, R.M. Marcé, Presence of pharmaceuticals and hormones in waters from sewage treatment plants, Water. Air. Soil Pollut. 217 (2011) 267–281. doi:10.1007/s11270-010-0585-8.

[23] A. Mousa, C. Basheer, A. Rahman Al-Arfaj, Application of electro-enhanced solid-phase microextraction for determination of phthalate esters and bisphenol A in blood and seawater samples, Talanta. 115 (2013) 308–313. doi:10.1016/j.talanta.2013.05.011.

[24] G.C.C. Yang, C.H. Yen, C.L. Wang, Monitoring and removal of residual phthalate esters and pharmaceuticals in the drinking water of Kaohsiung City, Taiwan, J. Hazard. Mater. 277 (2014) 53–61. doi:10.1016/j.jhazmat.2014.03.005.

[25] G. Prokůpková, K. Holadová, J. Poustka, J. Hajšlová, Development of a solid-phase microextraction method for the determination of phthalic acid esters in water, Anal. Chim. Acta. 457 (2002) 211–223. doi:10.1016/S0003-2670(02)00020-X.

[26] T.E. Félix-Cañedo, J.C. Durán-Álvarez, B. Jiménez-Cisneros, The occurrence and distribution of a group of organic micropollutants in Mexico City's water sources, Sci. Total Environ. 454–455 (2013) 109–118. doi:10.1016/j.scitotenv.2013.02.088.

[27] H.S. Shin, C.H. Park, S.J. Park, H. Pyo, Sensitive determination of bisphenol A in environmental water by gas chromatography with nitrogen-phosphorus detection after cyanomethylation, J. Chromatogr. A. 912 (2001) 119–125. doi:10.1016/S0021-9673(01)00570-2.

[28] X. Li, G.G. Ying, H.C. Su, X.B. Yang, L. Wang, Simultaneous determination and assessment of 4-nonylphenol, bisphenol A and triclosan in tap water, bottled water and baby bottles, Environ. Int. 36 (2010) 557–562. doi:10.1016/j.envint.2010.04.009.

[29] A. Belfroid, M. Van Velzen, B. Van der Horst, D. Vethaak, Occurrence of bisphenol A in surface water and uptake in fish: Evaluation of field measurements, Chemosphere. 49 (2002) 97–103. doi:10.1016/S0045-6535(02)00157-1.

[30] S.D. Kim, J. Cho, I.S. Kim, B.J. Vanderford, S.A. Snyder, Occurrence and removal of pharmaceuticals and endocrine disruptors in South Korean surface, drinking, and waste waters, Water Res. 41 (2007) 1013–1021. doi:10.1016/J.WATRES.2006.06.034.

[31] C. Basheer, H.K. Lee, K.S. Tan, Endocrine disrupting alkylphenols and bisphenol-A in coastal waters and supermarket seafood from Singapore, Mar. Pollut. Bull. 48 (2004) 1161–1167. doi:10.1016/S0213-9111(00)71916-9.

[32] D. Gao, Z. Li, Z. Wen, N. Ren, Occurrence and fate of phthalate esters in full-scale domestic wastewater treatment plants and their impact on receiving waters along the Songhua River in

China, Chemosphere. 95 (2014) 24–32. doi:10.1016/j.chemosphere.2013.08.009.
[33] X. Zheng, B.-T. Zhang, Y. Teng, Distribution of phthalate acid esters in lakes of Beijing and its relationship with anthropogenic activities., Sci. Total Environ. 476–477 (2014) 107–13. doi:10.1016/j.scitotenv.2013.12.111.
[34] W. He, N. Qin, X. Kong, W. Liu, Q. He, H. Ouyang, et al., Spatio-temporal distributions and the ecological and health risks of phthalate esters (PAEs) in the surface water of a large, shallow Chinese lake, Sci. Total Environ. 461–462 (2013) 672–680. doi:10.1016/j.scitotenv.2013.05.049.
[35] M.J. Teil, M. Blanchard, C. Dargnat, K. Larcher-Tiphagne, M. Chevreuil, Occurence of phthalate diesters in rivers of the Paris district (France), Hydrol. Process. 21 (2007) 2515–2525. doi:10.1002/hyp.6484.
[36] D.W. Kolpin, E.T. Furlong, M.T. Meyer, E.M. Thurman, S.D. Zaugg, L.B. Barber, et al., Pharmaceuticals, hormones, and other organic wastewater contaminants in U.S. streams, 1999-2000: A national reconnaissance, Environ. Sci. Technol. 36 (2002) 1202–1211. doi:10.1021/es011055j.
[37] J.H. Kang, F. Kondo, Bisphenol A in the Surface Water and Freshwater Snail Collected from Rivers Around a Secure Landfill, Bull. Environ. Contam. Toxicol. 76 (2006) 113–118. doi:10.1007/s00128-005-0896-4.
[38] *,† Toshinari Suzuki, ‡ Yoshio Nakagawa, ‡ Ichiro Takano, ‡ and Kumiko Yaguchi, K. Yasuda†, Environmental Fate of Bisphenol A and Its Biological Metabolites in River Water and Their Xeno-estrogenic Activity, (2004). doi:10.1021/ES030576Z.
[39] G.R. Boyd, J.M. Palmeri, S. Zhang, D.A. Grimm, Pharmaceuticals and personal care products (PPCPs) and endocrine disrupting chemicals (EDCs) in stormwater canals and Bayou St. John in New Orleans, Louisiana, USA, Sci. Total Environ. 333 (2004) 137–148. doi:10.1016/j.scitotenv.2004.03.018.
[40] R. Céspedes, S. Lacorte, A. Ginebreda, D. Barceló, Chemical monitoring and occurrence of alkylphenols, alkylphenol ethoxylates, alcohol ethoxylates, phthalates and benzothiazoles in sewage treatment plants and receiving waters along the ter River basin (Catalonia, N. E. Spain), in: Anal. Bioanal. Chem., Springer-Verlag, 2006: pp. 992–1000. doi:10.1007/s00216-006-0448-8.
[41] H. Chang, S. Wu, J. Hu, M. Asami, S. Kunikane, Trace analysis of androgens and progestogens in environmental waters by ultra-performance liquid chromatography-electrospray tandem mass spectrometry, J. Chromatogr. A. 1195 (2008) 44–51. doi:10.1016/j.chroma.2008.04.055.
[42] S. Kleywegt, V. Pileggi, P. Yang, C. Hao, X. Zhao, C. Rocks, et al., Pharmaceuticals, hormones and bisphenol A in untreated source and finished drinking water in Ontario, Canada - Occurrence and treatment efficiency, Sci. Total Environ. 409 (2011) 1481–1488. doi:10.1016/j.scitotenv.2011.01.010.
[43] V.A. Santhi, N. Sakai, E.D. Ahmad, A.M. Mustafa, Occurrence of bisphenol A in surface water, drinking water and plasma from Malaysia with exposure assessment from consumption of drinking water, Sci. Total Environ. 427–428 (2012) 332–338. doi:10.1016/j.scitotenv.2012.04.041.
[44] A. Colin, C. Bach, C. Rosin, J.-F. Munoz, X. Dauchy, Is Drinking Water a Major Route of Human Exposure to Alkylphenol and Bisphenol Contaminants in France?, Arch. Environ. Contam. Toxicol. 66 (2014) 86–99. doi:10.1007/s00244-013-9942-0.
[45] M.T. Das, P. Ghosh, I.S. Thakur, Intake estimates of phthalate esters for South Delhi population based on exposure media assessment, Environ. Pollut. 189 (2014) 118–125. doi:10.1016/J.ENVPOL.2014.02.021.
[46] Y.B. Luo, Q.W. Yu, B.F. Yuan, Y.Q. Feng, Fast microextraction of phthalate acid esters from beverage, environmental water and perfume samples by magnetic multi-walled carbon nanotubes, Talanta. 90 (2012) 123–131. doi:10.1016/j.talanta.2012.01.015.
[47] X. Wu, H. Hong, X. Liu, W. Guan, L. Meng, Y. Ye, et al., Graphene-dispersive solid-phase

extraction of phthalate acid esters from environmental water, Sci. Total Environ. 444 (2013) 224–230. doi:10.1016/j.scitotenv.2012.11.060.

[48] R. Loos, G. Hanke, G. Umlauf, S.J. Eisenreich, LC-MS-MS analysis and occurrence of octyl- and nonylphenol, their ethoxylates and their carboxylates in Belgian and Italian textile industry, waste water treatment plant effluents and surface waters, Chemosphere. 66 (2007) 690–699. doi:10.1016/j.chemosphere.2006.07.060.

[49] C.C. Lee, L.Y. Jiang, Y.L. Kuo, C.Y. Hsieh, C.S. Chen, C.J. Tien, The potential role of water quality parameters on occurrence of nonylphenol and bisphenol A and identification of their discharge sources in the river ecosystems, Chemosphere. 91 (2013) 904–911. doi:10.1016/j.chemosphere.2013.02.006.

[50] X.L. Cao, J. Corriveau, Determination of bisphenol a in water by isotope dilution headspace solid-phase microextraction and gas chromatography/mass spectrometry without derivatization, J. AOAC Int. 91 (2008) 622–629. doi:10.1016/j.chroma.2007.11.095.

[51] D.A. Alvarez, K.A. Maruya, N.G. Dodder, W. Lao, E.T. Furlong, K.L. Smalling, Occurrence of contaminants of emerging concern along the California coast (2009 – 10) using passive sampling devices, Mar. Pollut. Bull. 81 (2014) 347–354. doi:10.1016/j.marpolbul.2013.04.022.

[52] Z. Fan, S. Wu, H. Chang, J. Hu, Behaviors of Glucocorticoids, Androgens and Progestogens in a Municipal Sewage Treatment Plant: Comparison to Estrogens, Environ. Sci. Technol. 45 (2011) 2725–2733. doi:10.1021/es103429c.

[53] F. Zeng, K. Cui, Z. Xie, M. Liu, Y. Li, Y. Lin, et al., Occurrence of phthalate esters in water and sediment of urban lakes in a subtropical city, Guangzhou, South China, Environ. Int. 34 (2008) 372–380. doi:10.1016/j.envint.2007.09.002.

[54] Y. Liu, Z. Chen, J. Shen, Occurrence and removal characteristics of phthalate esters from typical water sources in northeast China, J. Anal. Methods Chem. 2013 (2013) 419349. doi:10.1155/2013/419349.

[55] S. Net, D. Dumoulin, R. El-Osmani, S. Rabodonirina, B. Ouddane, Case study of PAHs, Me-PAHs, PCBs, phthalates and pesticides contamination in the Somme River water, France, Int. J. Environ. Res. 8 (2014) 1159–1170. https://ijer.ut.ac.ir/article_809_24fc56c34d8b0f38ffc5b152c55c28bb.pdf (accessed November 8, 2018).

[56] A.J. Al Khatib, M. Muhammad, A. Adamu, ANALYSIS OF PHTHALATE PLASTICIZER IN JORDANIAN BOTTLED WATERS BY LIQUID CHROMATOGRAPHY-TANDEM MASS SPECTROPHOTOMETRY (LC-MS / MS), Eur. Sci. Journal, ESJ. 10 (2014) 271–282. https://eujournal.org/index.php/esj/article/view/3596 (accessed March 7, 2018).

[57] C. Dargnat, M.-J. Teil, M. Chevreuil, M. Blanchard, Phthalate removal throughout wastewater treatment plant: Case study of Marne Aval station (France), Sci. Total Environ. 407 (2009) 1235–1244. doi:10.1016/J.SCITOTENV.2008.10.027.

[58] D.A. Alvarez, P.E. Stackelberg, J.D. Petty, J.N. Huckins, E.T. Furlong, S.D. Zaugg, et al., Comparison of a novel passive sampler to standard water-column sampling for organic contaminants associated with wastewater effluents entering a New Jersey stream, Chemosphere. 61 (2005) 610–622. doi:10.1016/j.chemosphere.2005.03.023.

[59] E. Vulliet, L. Wiest, R. Baudot, M.F. Grenier-Loustalot, Multi-residue analysis of steroids at sub-ng/L levels in surface and ground-waters using liquid chromatography coupled to tandem mass spectrometry, J. Chromatogr. A. 1210 (2008) 84–91. doi:10.1016/j.chroma.2008.09.034.

[60] M.P. Fernandez, M.G. Ikonomou, I. Buchanan, An assessment of estrogenic organic contaminants in Canadian wastewaters, Sci. Total Environ. 373 (2007) 250–269. doi:10.1016/j.scitotenv.2006.11.018.

[61] H.M. Kuch, K. Ballschmiter, Determination of endocrine-disrupting phenolic compounds and estrogens in surface and drinking water by HRGC-(NCI)-MS in the picogram per liter range, Environ. Sci. Technol. 35 (2001) 3201–3206. doi:10.1021/es010034m.

[62] C. Baronti, R. Curini, G. D'Ascenzo, A. Di Corcia, A. Gentili, R. Samperi, Monitoring natural and

synthetic estrogens at activated sludge sewage treatment plants and in a receiving river water, Environ. Sci. Technol. 34 (2000) 5059–5066. doi:10.1021/es001359q.

[63] T. Wu, C. Wang, X. Wang, H. Xiao, Q. Ma, Q. Zhang, Comparison of UPLC and HPLC for Analysis of 12 Phthalates, Chromatographia. 68 (2008) 803–806. doi:10.1365/s10337-008-0788-y.

[64] M.I. Churchwell, N.C. Twaddle, L.R. Meeker, D.R. Doerge, Improving LC-MS sensitivity through increases in chromatographic performance: Comparisons of UPLC-ES/MS/MS to HPLC-ES/MS/MS, J. Chromatogr. B Anal. Technol. Biomed. Life Sci. 825 (2005) 134–143. doi:10.1016/j.jchromb.2005.05.037.

[65] P.B. Fayad, M. Prévost, S. Sauvé, On-line solid-phase extraction coupled to liquid chromatography tandem mass spectrometry optimized for the analysis of steroid hormones in urban wastewaters, Talanta. 115 (2013) 349–360. doi:10.1016/j.talanta.2013.05.038.

[66] S.X.L. Goh, A. Duarah, L. Zhang, S.A. Snyder, H.K. Lee, Online solid phase extraction with liquid chromatography–tandem mass spectrometry for determination of estrogens and glucocorticoids in water, J. Chromatogr. A. 1465 (2016) 9–19. doi:10.1016/j.chroma.2016.08.040.

[67] R. Loos, GIVES LEVELS - Analytical Methods for the new proposed Priority Substances of the European Water Framework Directive (WFD), JRC Tech. Rep. (2012) 71. doi:10.2788/51497.

[68] A. Andrade-Eiroa, M. Canle, V. Leroy-Cancellieri, V. Cerdà, Solid-phase extraction of organic compounds: A critical review (Part I), TrAC - Trends Anal. Chem. 80 (2016) 641–654. doi:10.1016/j.trac.2015.08.015.

[69] The European Parliament and the Council of the European Union, Commission Decision 2002/657/EC implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results, 96/23/Ec Comm. Decis. (2002) 29.

[70] O. Magnusson, Eurachem Guide: The Fitness for Purpose of Analytical Methods – A Laboratory Guide to Method Validation and Related Topics, 2014. doi:978-91-87461-59-0.

[71] A. Kruve, R. Rebane, K. Kipper, M.-L. Oldekop, H. Evard, K. Herodes, et al., Tutorial review on validation of liquid chromatography–mass spectrometry methods: Part I, Anal. Chim. Acta. 870 (2015) 29–44. doi:10.1016/j.aca.2015.02.017.

[72] A. Kruve, R. Rebane, K. Kipper, M.-L. Oldekop, H. Evard, K. Herodes, et al., Tutorial review on validation of liquid chromatography–mass spectrometry methods: Part II, Anal. Chim. Acta. 870 (2015) 8–28. doi:10.1016/j.aca.2015.02.016.

[73] S. Huysman, L. Van Meulebroek, F. Vanryckeghem, H. Van Langenhove, K. Demeestere, L. Vanhaecke, Development and validation of an ultra-high performance liquid chromatographic high resolution Q-Orbitrap mass spectrometric method for the simultaneous determination of steroidal endocrine disrupting compounds in aquatic matrices, Anal. Chim. Acta. 984 (2017). doi:10.1016/j.aca.2017.07.001.

[74] W.J. Griffiths, Y. Wang, Mass spectrometry: From proteomics to metabolomics and lipidomics, Chem. Soc. Rev. 38 (2009) 1882–1896. doi:10.1039/b618553n.

[75] T. Köcher, R. Swart, K. Mechtler, Ultra-high-pressure RPLC hyphenated to an LTQ-orbitrap velos reveals a linear relation between peak capacity and number of identified peptides, Anal. Chem. 83 (2011) 2699–2704. doi:10.1021/ac103243t.

[76] M.J. Benotti, R.A. Trenholm, B.J. Vanderford, J.C. Holady, B.D. Stanford, S.A. Snyder, Pharmaceuticals and endocrine disrupting compounds in U.S. drinking water, Environ. Sci. Technol. 43 (2009) 597–603. doi:10.1021/es801845a.

[77] J. Cotton, F. Leroux, S. Broudin, M. Poirel, B. Corman, C. Junot, et al., Development and validation of a multiresidue method for the analysis of more than 500 pesticides and drugs in water based on on-line and liquid chromatography coupled to high resolution mass spectrometry, Water Res. 104 (2016) 20–27. doi:10.1016/j.watres.2016.07.075.

[78] S. Huysman, L. Van Meulebroek, O. Janssens, F. Vanryckeghem, H. Van Langenhove, K. Demeestere, et al., Targeted quantification and untargeted screening of alkylphenols, bisphenol A and phthalates in aquatic matrices using ultra-high-performance liquid

chromatography coupled to hybrid Q-Orbitrap mass spectrometry, Anal. Chim. Acta. 1049 (2019) 141–151. doi:10.1016/j.aca.2018.10.045.

[79] S. Huysman, L. Van Meulebroek, O. Janssens, F. Vanryckeghem, H. Van Langenhove, K. Demeestere, et al., Targeted quantification and untargeted screening of alkylphenols, bisphenol A and phthalates in aquatic matrices using ultra-high-performance liquid chromatography coupled to hybrid Q-Orbitrap mass spectrometry, Anal. Chim. Acta. 1049 (2019) 141–151. doi:10.1016/j.aca.2018.10.045.

[80] F. Hernández, M. Ibáñez, A.M. Botero-Coy, R. Bade, M.C. Bustos-López, J. Rincón, et al., LC-QTOF MS screening of more than 1,000 licit and illicit drugs and their metabolites in wastewater and surface waters from the area of Bogotá, Colombia, Anal. Bioanal. Chem. 407 (2015) 6405–6416. doi:10.1007/s00216-015-8796-x.

[81] C. Moschet, A. Piazzoli, H. Singer, J. Hollender, Alleviating the reference standard dilemma using a systematic exact mass suspect screening approach with liquid chromatography-high resolution mass spectrometry, Anal. Chem. 85 (2013) 10312–10320. doi:10.1021/ac4021598.

[82] E.L. Schymanski, H.P. Singer, J. Slobodnik, I.M. Ipolyi, P. Oswald, M. Krauss, et al., Non-target screening with high-resolution mass spectrometry: Critical review using a collaborative trial on water analysis, Anal. Bioanal. Chem. 407 (2015) 6237–6255. doi:10.1007/s00216-015-8681-7.

[83] R.M.A. Sjerps, D. Vughs, J.A. van Leerdam, T.L. ter Laak, A.P. van Wezel, Data-driven prioritization of chemicals for various water types using suspect screening LC-HRMS, Water Res. 93 (2016) 254–264. doi:10.1016/j.watres.2016.02.034.

[84] E.L. Schymanski, J. Jeon, R. Gulde, K. Fenner, M. Ruff, H.P. Singer, et al., Identifying small molecules via high resolution mass spectrometry: Communicating confidence, Environ. Sci. Technol. 48 (2014) 2097–2098. doi:10.1021/es5002105.

[85] A. Martin, C. Margoum, J. Randon, M. Coquery, Silicone rubber selection for passive sampling of pesticides in water, Talanta. 160 (2016) 306–313. doi:10.1016/j.talanta.2016.07.019.

[86] F. Smedes, R.W. Geertsma, T. Van Der Zande, K. Booij, Polymer-water partition coefficients of hydrophobic compounds for passive sampling: Application of cosolvent models for validation, Environ. Sci. Technol. 43 (2009) 7047–7054. doi:10.1021/es9009376.

[87] A. Charriau, S. Lissalde, G. Poulier, N. Mazzella, R. Buzier, G. Guibaud, Overview of the Chemcatcher® for the passive sampling of various pollutants in aquatic environments Part A: Principles, calibration, preparation and analysis of the sampler, Talanta. 148 (2016) 556–571. doi:10.1016/J.TALANTA.2015.06.064.

[88] S. Lissalde, A. Charriau, G. Poulier, N. Mazzella, R. Buzier, G. Guibaud, Overview of the Chemcatcher® for the passive sampling of various pollutants in aquatic environments Part B: Field handling and environmental applications for the monitoring of pollutants and their biological effects, Talanta. 148 (2016) 572–582. doi:10.1016/j.talanta.2015.06.076.

[89] D. a Alvarez, J.D. Petty, J.N. Huckins, T.L. Jones-Lepp, D.T. Getting, J.P. Goddard, et al., Development of a passive, in situ, integrative sampler for hydrophilic organic contaminants in aquatic environments., Environ. Toxicol. Chem. 23 (2004) 1640–1648. doi:10.1897/03-603.

[90] K. Booij, S. Chen, Review of atrazine sampling by polar organic chemical integrative samplers and Chemcatcher, Environ. Toxicol. Chem. 37 (2018) 1786–1798. doi:10.1002/etc.4160.

[91] C. Metcalfe, M.E. Hoque, T. Sultana, C. Murray, P. Helm, S. Kleywegt, Monitoring for contaminants of emerging concern in drinking water using POCIS passive samplers, Environ. Sci. Process. Impacts. 16 (2014) 473. doi:10.1039/c3em00508a.

[92] L. Ahrens, A. Daneshvar, A.E. Lau, J. Kreuger, Characterization of five passive sampling devices for monitoring of pesticides in water, J. Chromatogr. A. 1405 (2015) 1–11. doi:10.1016/j.chroma.2015.05.044.

[93] C. Tixier, C. Miège, H. Budzinski, J. Brant, N. Mazzella, I. Allan, et al., Position paper on passive sampling techniques for the monitoring of contaminants in the aquatic environment – Achievements to date and perspectives, Trends Environ. Anal. Chem. 8 (2015) 20–26. doi:10.1016/j.teac.2015.07.001.

[94] European Commission, Common implementation strategy for the Water Framework Directive (2000/60/EC). Guidance on surface water chemical monitoring under the Water Framework Directive., 2009. https://circabc.europa.eu/sd/a/ba1fa997-a5a9-44b1-8016-f14751a86f32/CIS Guidance Article 4.7 - Draft 1.pdf (accessed February 25, 2019).

[95] CD 2013/39/EU, Directive 2013/39/EU of the European Parliament and of the Council of 12 August 2013, 2013.

[96] O.J. Pozo, P. Van Eenoo, K. Deventer, S. Grimalt, J. V. Sancho, F. Hernández, et al., Collision-induced dissociation of 3-keto anabolic steroids and related compounds after electrospray ionization. Considerations for structural elucidation, Rapid Commun. Mass Spectrom. 22 (2008) 4009–4024. doi:10.1002/rcm.3823.

[97] M. Thevis, A.A. Makarov, S. Horning, W. Schänzer, Mass spectrometry of stanozolol and its analogues using electrospray ionization and collision-induced dissociation with quadrupole-linear ion trap and linear ion trap-orbitrap hybrid mass analyzers, in: Rapid Commun. Mass Spectrom., John Wiley & Sons, Ltd, 2005: pp. 3369–3378. doi:10.1002/rcm.2204.

[98] Ó.J. Pozo, P. Van Eenoo, K. Deventer, L. Lootens, W. Van Thuyne, M.K. Parr, et al., Detection and characterization of a new metabolite of 17α- methyltestosterone, Drug Metab. Dispos. 37 (2009) 2153–2162. doi:10.1124/dmd.109.028373.

[99] G.M. Randazzo, D. Tonoli, S. Hambye, D. Guillarme, F. Jeanneret, A. Nurisso, et al., Prediction of retention time in reversed-phase liquid chromatography as a tool for steroid identification, Anal. Chim. Acta. 916 (2016) 8–16. doi:10.1016/j.aca.2016.02.014.

[100] J.J.J. van der Hooft, L. Ridder, M.P. Barrett, K.E. V. Burgess, Enhanced Acylcarnitine Annotation in High-Resolution Mass Spectrometry Data: Fragmentation Analysis for the Classification and Annotation of Acylcarnitines, Front. Bioeng. Biotechnol. 3 (2015) 26. doi:10.3389/fbioe.2015.00026.

[101] P. Gago-Ferrero, E.L. Schymanski, A.A. Bletsou, R. Aalizadeh, J. Hollender, N.S. Thomaidis, Extended Suspect and Non-Target Strategies to Characterize Emerging Polar Organic Contaminants in Raw Wastewater with LC-HRMS/MS, Environ. Sci. Technol. 49 (2015) 12333–12341. doi:10.1021/acs.est.5b03454.

[102] T. Kind, O. Fiehn, Seven Golden Rules for heuristic filtering of molecular formulas obtained by accurate mass spectrometry, BMC Bioinformatics. 8 (2007) 105. doi:10.1186/1471-2105-8-105.

[103] M. Meringer, S. Reinker, J. Zhang, A. Muller, MS / MS Data Improves Automated Determination of Molecular Formulas by Mass Spectrometry, MATCH Commun. Math. Comput. Chem. 65 (2011) 259–290. https://www.researchgate.net/publication/225022489 (accessed February 26, 2019).

[104] H. Horai, M. Arita, S. Kanaya, Y. Nihei, T. Ikeda, K. Suwa, et al., MassBank: A public repository for sharing mass spectral data for life sciences, J. Mass Spectrom. 45 (2010) 703–714. doi:10.1002/jms.1777.

[105] M.J. Andrés-Costa, V. Andreu, Y. Picó, Liquid chromatography–mass spectrometry as a tool for wastewater-based epidemiology: Assessing new psychoactive substances and other human biomarkers, TrAC - Trends Anal. Chem. 94 (2017) 21–38. doi:10.1016/j.trac.2017.06.012.

[106] W.J. Weber, P.M. McGinley, L.E. Katz, A Distributed Reactivity Model for Sorption by Soils and Sediments. 1. Conceptual Basis and Equilibrium Assessments, Environ. Sci. Technol. 26 (1992) 1955–1962. doi:10.1021/es00034a012.

[107] P.M. McGinley, L.E. Katz, W.J. Weber, A Distributed Reactivity Model for Sorption by Soils and Sediments. 2. Multicomponent Systems and Competitive Effects, Environ. Sci. Technol. 27 (1993) 1524–1531. doi:10.1021/es00045a006.

[108] P.M. McGinley, L.E. Katz, W.J. Weber, Competitive sorption and displacement of hydrophobic organic contaminants in saturated subsurface soil systems, Water Resour. Res. 32 (1996) 3571–3577. doi:10.1029/96WR02694.

[109] J.J. Pignatello, Soil organic matter as a nanoporous sorbent of organic pollutants, Adv. Colloid

Interface Sci. 76–77 (1998) 445–467. doi:10.1016/S0001-8686(98)00055-4.
[110] J.J. Pignatello, Interactions of Anthropogenic Organic Chemicals with Natural Organic Matter and Black Carbon in Environmental Particles, in: Biophys. Process. Anthropog. Org. Compd. Environ. Syst., John Wiley & Sons, Inc., Hoboken, NJ, USA, 2011: pp. 1–50. doi:10.1002/9780470944479.ch1.
[111] B. Xing, J.J. Pignatello, Dual-mode sorption of low-polarity compounds in glassy poly(vinyl chloride) and soil organic matter, Environ. Sci. Technol. 31 (1997) 792–799. doi:10.1021/es960481f.
[112] D. Ju, T.M. Young, Effects of competitor and natural organic matter characteristics on the equilibrium sorption of 1,2-dichlorobenzene in soil and shale, Environ. Sci. Technol. 38 (2004) 5863–5870. doi:10.1021/es049668u.
[113] B. Xing, J.J. Pignatello, Competitive sorption between 1,3-dichlorobenzene or 2,4-dichlorophenol and natural aromatic acids in soil organic matter, Environ. Sci. Technol. 32 (1998) 614–619. doi:10.1021/es9704646.
[114] X. Wang, S. Tao, B. Xing, Sorption and Competition of Aromatic Compounds and Humic Acid on Multiwalled Carbon Nanotubes, Environ. Sci. Technol. 43 (2009) 6214–6219. doi:10.1021/es901062t.
[115] J.K. Challis, M.L. Hanson, C.S. Wong, Pharmaceuticals and pesticides archived on polar passive sampling devices can be stable for up to 6 years, Environ. Toxicol. Chem. 37 (2018) 762–767. doi:10.1002/etc.4012.
[116] J.C. Carlson, J.K. Challis, M.L. Hanson, C.S. Wong, Stability of pharmaceuticals and other polar organic compounds stored on polar organic chemical integrative samplers and solid-phase extraction cartridges, Environ. Toxicol. Chem. 32 (2013) 337–344. doi:10.1002/etc.2076.
[117] C.E. Chen, H. Zhang, G.G. Ying, K.C. Jones, Evidence and recommendations to support the use of a novel passive water sampler to quantify antibiotics in wastewaters, Environ. Sci. Technol. 47 (2013) 13587–13593. doi:10.1021/es402662g.
[118] J.K. Challis, M.L. Hanson, C.S. Wong, Development and Calibration of an Organic-Diffusive Gradients in Thin Films Aquatic Passive Sampler for a Diverse Suite of Polar Organic Contaminants, Anal. Chem. 88 (2016) 10583–10591. doi:10.1021/acs.analchem.6b02749.
[119] N. Morin, C. Miège, M. Coquery, J. Randon, Chemical calibration, performance, validation and applications of the polar organic chemical integrative sampler (POCIS) in aquatic environments, TrAC Trends Anal. Chem. 36 (2012) 144–175. doi:10.1016/J.TRAC.2012.01.007.
[120] R. Bade, L. Bijlsma, A.-K. McCall, F.Y. Lai, J. Kinyua, P.K. Thai, et al., Critical review on the stability of illicit drugs in sewers and wastewater samples, Water Res. 88 (2015) 933–947. doi:10.1016/j.watres.2015.10.040.
[121] A.L.N. van Nuijs, S. Castiglioni, I. Tarcomnicu, C. Postigo, M.L. de Alda, H. Neels, et al., Illicit drug consumption estimations derived from wastewater analysis: A critical review, Sci. Total Environ. 409 (2011) 3564–3577. doi:10.1016/J.SCITOTENV.2010.05.030.
[122] S. Castiglioni, K. V. Thomas, B. Kasprzyk-Hordern, L. Vandam, P. Griffiths, Testing wastewater to detect illicit drugs: State of the art, potential and research needs, Sci. Total Environ. 487 (2014) 613–620. doi:10.1016/j.scitotenv.2013.10.034.
[123] I. González-Mariño, R. Rodil, I. Barrio, R. Cela, J.B. Quintana, Wastewater-Based Epidemiology as a New Tool for Estimating Population Exposure to Phthalate Plasticizers, Environ. Sci. Technol. 51 (2017) 3902–3910. doi:10.1021/acs.est.6b05612.
[124] C.J. Banta-Green, A.J. Brewer, C. Ort, D.R. Helsel, J.R. Williams, J.A. Field, Using wastewater-based epidemiology to estimate drug consumption—Statistical analyses and data presentation, Sci. Total Environ. 568 (2016) 856–863. doi:10.1016/j.scitotenv.2016.06.052.
[125] M. Rico, M.J. Andrés-Costa, Y. Picó, Estimating population size in wastewater-based epidemiology. Valencia metropolitan area as a case study, J. Hazard. Mater. 323 (2017) 156–165. doi:10.1016/J.JHAZMAT.2016.05.079.
[126] C.M. de Jongh, P.J.F. Kooij, P. de Voogt, T.L. ter Laak, Screening and human health risk

assessment of pharmaceuticals and their transformation products in Dutch surface waters and drinking water, Sci. Total Environ. 427–428 (2012) 70–77. doi:10.1016/j.scitotenv.2012.04.010.

[127] B. Vrana, I.J. Allan, R. Greenwood, G.A. Mills, E. Dominiak, K. Svensson, et al., Passive sampling techniques for monitoring pollutants in water, TrAC - Trends Anal. Chem. 24 (2005) 845–868. doi:10.1016/j.trac.2005.06.006.

[128] T. Katagi, Bioconcentration, bioaccumulation, and metabolism of pesticides in aquatic organisms, Rev. Environ. Contam. Toxicol. 204 (2010) 1–132. doi:10.1007/978-1-4419-1440-8_1.

[129] J.-P. Besse, O. Geffard, M. Coquery, Relevance and applicability of active biomonitoring in continental waters under the Water Framework Directive, TrAC Trends Anal. Chem. 36 (2012) 113–127. doi:10.1016/J.TRAC.2012.04.004.

[130] L. Jin, C. Gaus, L. Van Mourik, B.I. Escher, Applicability of passive sampling to bioanalytical screening of bioaccumulative chemicals in marine wildlife, Environ. Sci. Technol. 47 (2013) 7982–7988. doi:10.1021/es401014b.

[131] S.N. Schmidt, M. Holmstrup, K.E.C. Smith, P. Mayer, Passive dosing of polycyclic aromatic hydrocarbon (PAH) mixtures to terrestrial springtails: Linking mixture toxicity to chemical activities, equilibrium lipid concentrations, and toxic units, Environ. Sci. Technol. 47 (2013) 7020–7027. doi:10.1021/es3047813.

[132] N.M. Roden, J.M. Fiori, E.P. Hayes, D. Cragin, F.J. Mastrocco, V.J. D'Aco, et al., Human pharmaceuticals in US surface waters: A human health risk assessment, Regul. Toxicol. Pharmacol. 42 (2005) 296–312. doi:10.1016/j.yrtph.2005.05.005.

[133] V.L. Cunningham, S.P. Binks, M.J. Olson, Human health risk assessment from the presence of human pharmaceuticals in the aquatic environment, Regul. Toxicol. Pharmacol. 53 (2009) 39–45. doi:10.1016/j.yrtph.2008.10.006.

[134] K.E.C. Smith, N. Dom, R. Blust, P. Mayer, Controlling and maintaining exposure of hydrophobic organic compounds in aquatic toxicity tests by passive dosing, Aquat. Toxicol. 98 (2010) 15–24. doi:10.1016/j.aquatox.2010.01.007.

[135] T.H. Hutchinson, N. Shillabeer, M.J. Winter, D.B. Pickford, Acute and chronic effects of carrier solvents in aquatic organisms: A critical review, Aquat. Toxicol. 76 (2006) 69–92. doi:10.1016/j.aquatox.2005.09.008.

[136] B. Leoni, R. Bettinetti, S. Galassi, Sub-lethal effects of acetone on Daphnia magna, Ecotoxicology. 17 (2008) 199–205. doi:10.1007/s10646-007-0184-7.

[137] J. Neuwoehner, A. Tiehm, H. Hollert, A. Eisentraeger, A. Sagner, C. Brinkmann, HETEROCYCLIC COMPOUNDS: TOXIC EFFECTS USING ALGAE, DAPHNIDS, AND THE SALMONELLA/MICROSOME TEST TAKING METHODICAL QUANTITATIVE ASPECTS INTO ACCOUNT, Environ. Toxicol. Chem. 27 (2008) 1590. doi:10.1897/07-201.1.

Appendices

APPENDIX A - DEVELOPMENT AND VALIDATION OF AN ULTRA-HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC HIGH RESOLUTION Q-ORBITRAP MASS SPECTROMETRIC METHOD FOR THE SIMULTANEOUS DETERMINATION OF STEROIDAL ENDOCRINE DISRUPTING COMPOUNDS IN AQUATIC MATRICES

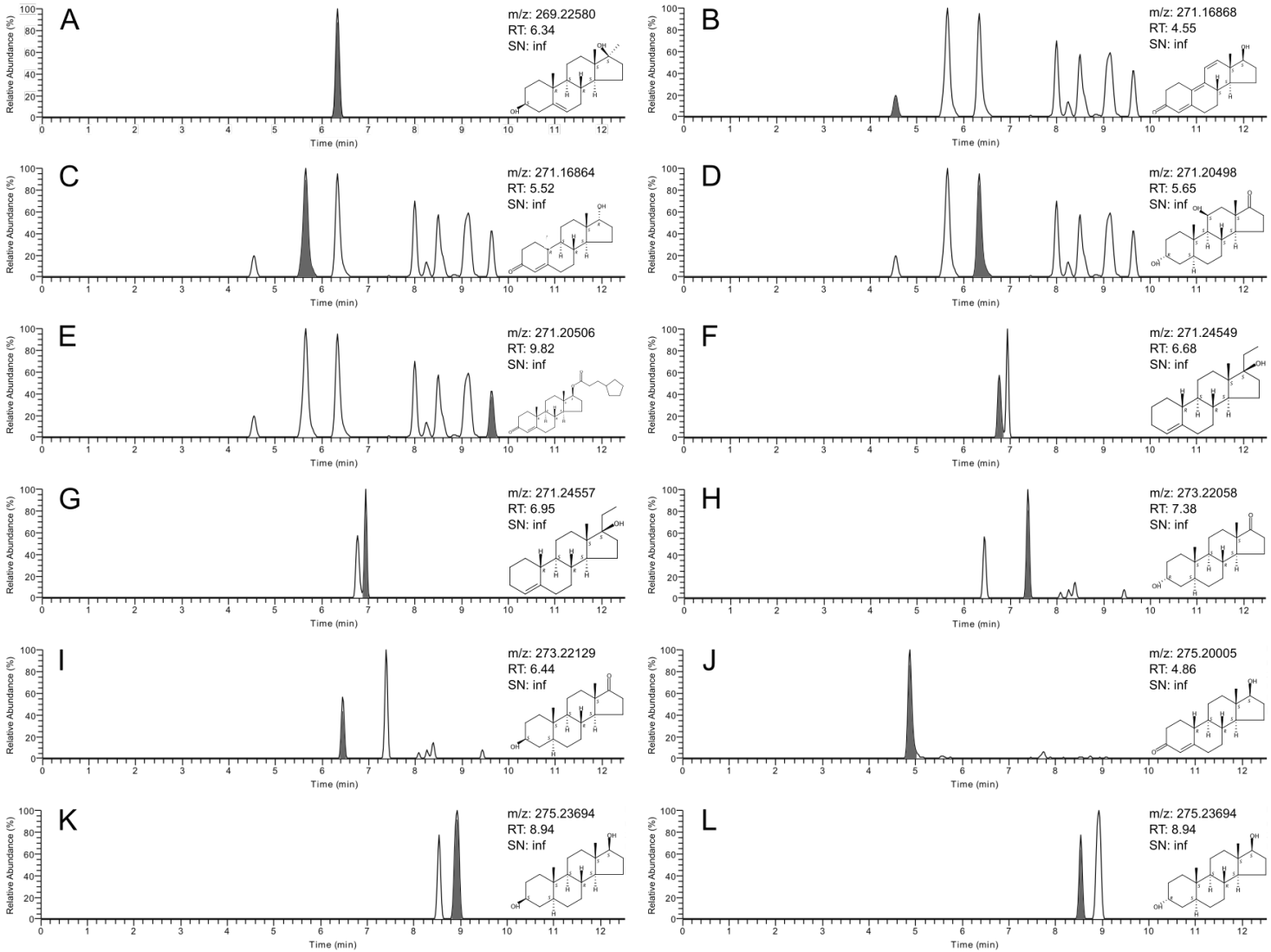


Figure A1. UHPLC-HR-Q-Orbitrap-MS chromatograms of the androgens included in this method (part I), i.e. (a) methandriol, (b) 17β-trenbolone, (c) 17α-trenbolone, (d), 11β-hydroxyandrosterone, (e) testosterone 17β-cypionate, (f) ethylestrenol, (g) 17β-dihydroandrosterone, (h) androsterone, (i) epi-androsterone, (j)19-nortestosterone, (k) 3α-androstanediol and (l) 3β-androstanediol.

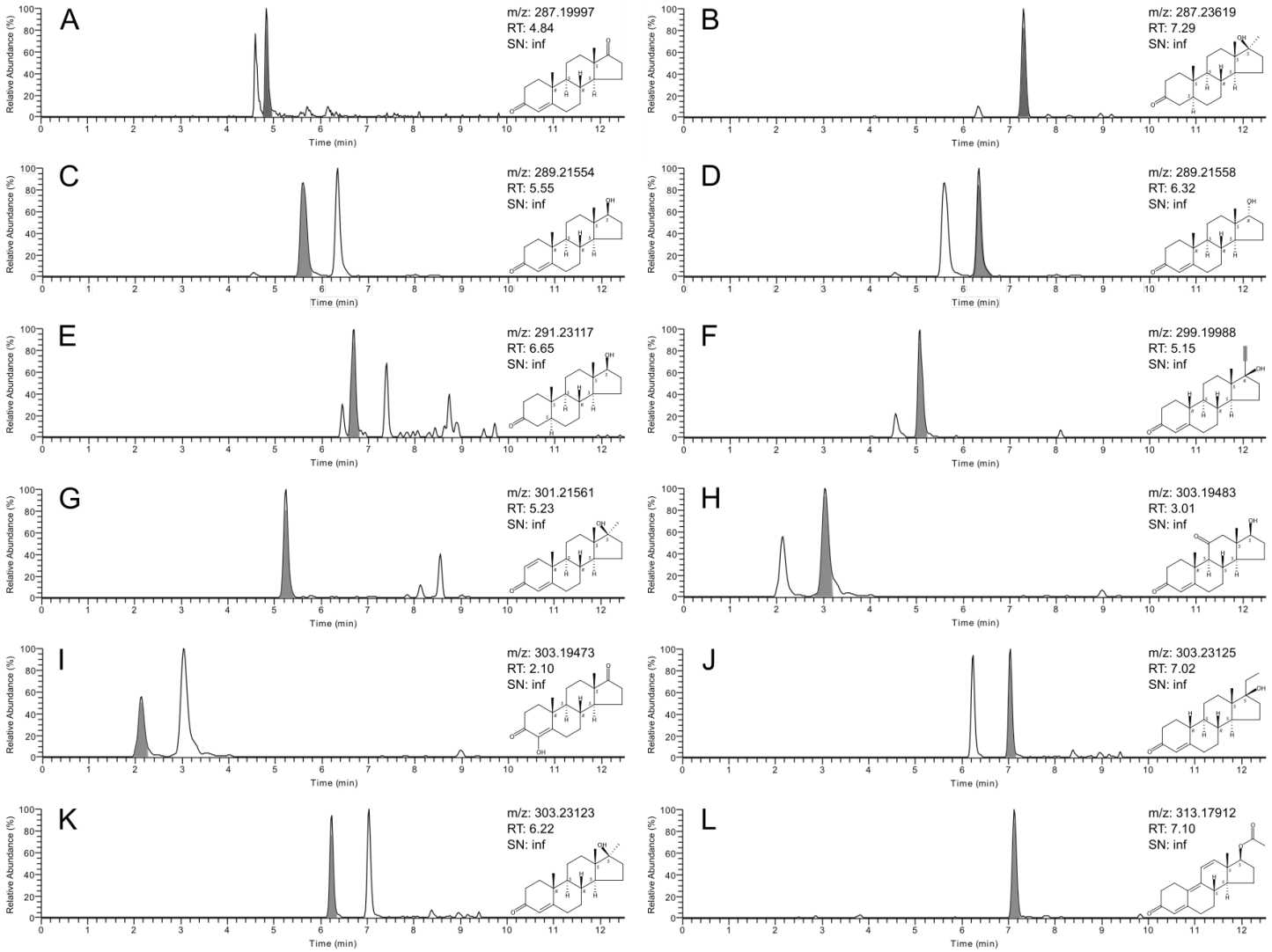


Figure A2. UHPLC-HR-Q-Orbitrap-MS chromatograms of the androgens included in this method (part II), i.e. (a) androstenedione, (b) mestanolone, (c) 17β-testosterone, (d) 17α-testosterone, (e) 5α- diydrotestosterone, (f) norethindrone, (g) methylboldenone, (h)11-ketotestosterone, (i) formestane, (j) norethandrolone, (k) methyltestosterone and (L) trenbolone acetate.

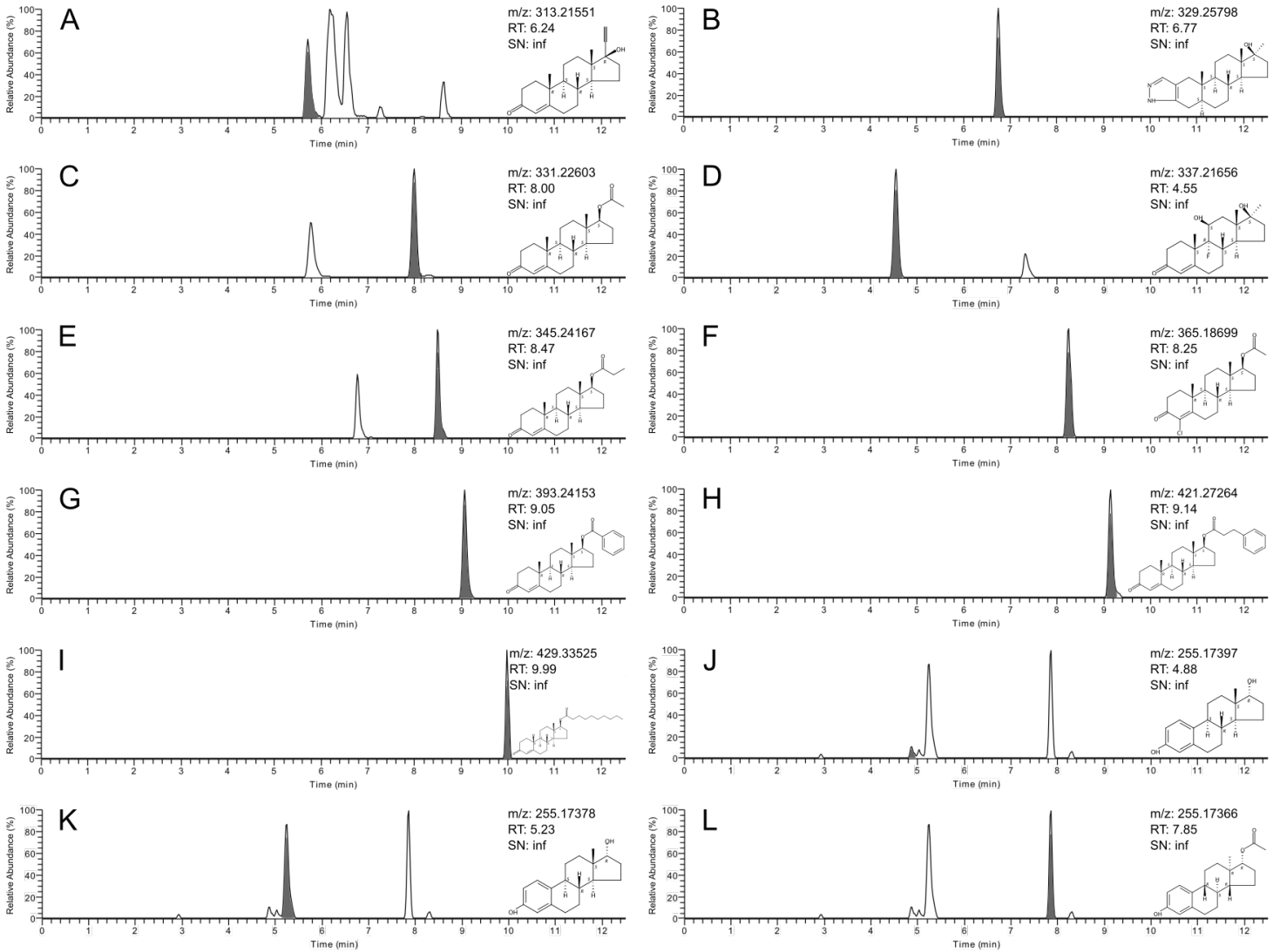


Figure A3. UHPLC-HR-Q-Orbitrap-MS chromatograms of the androgens included in this method (part III), i.e. (a) ethynyl testosterone, (b) stanozolol, (c) testosterone acetate, (d) fluoxymesterone, (e) testosterone propionate, (f) chlorotestosterone aceate, (h) testosterone benzoate and (i) 19-nortestosterone-17-decanoate, and oestrogens (part I), i.e. (j) 17β-estradiol, (k) 17α-estradiol and (l) estradiol-17-acetate.

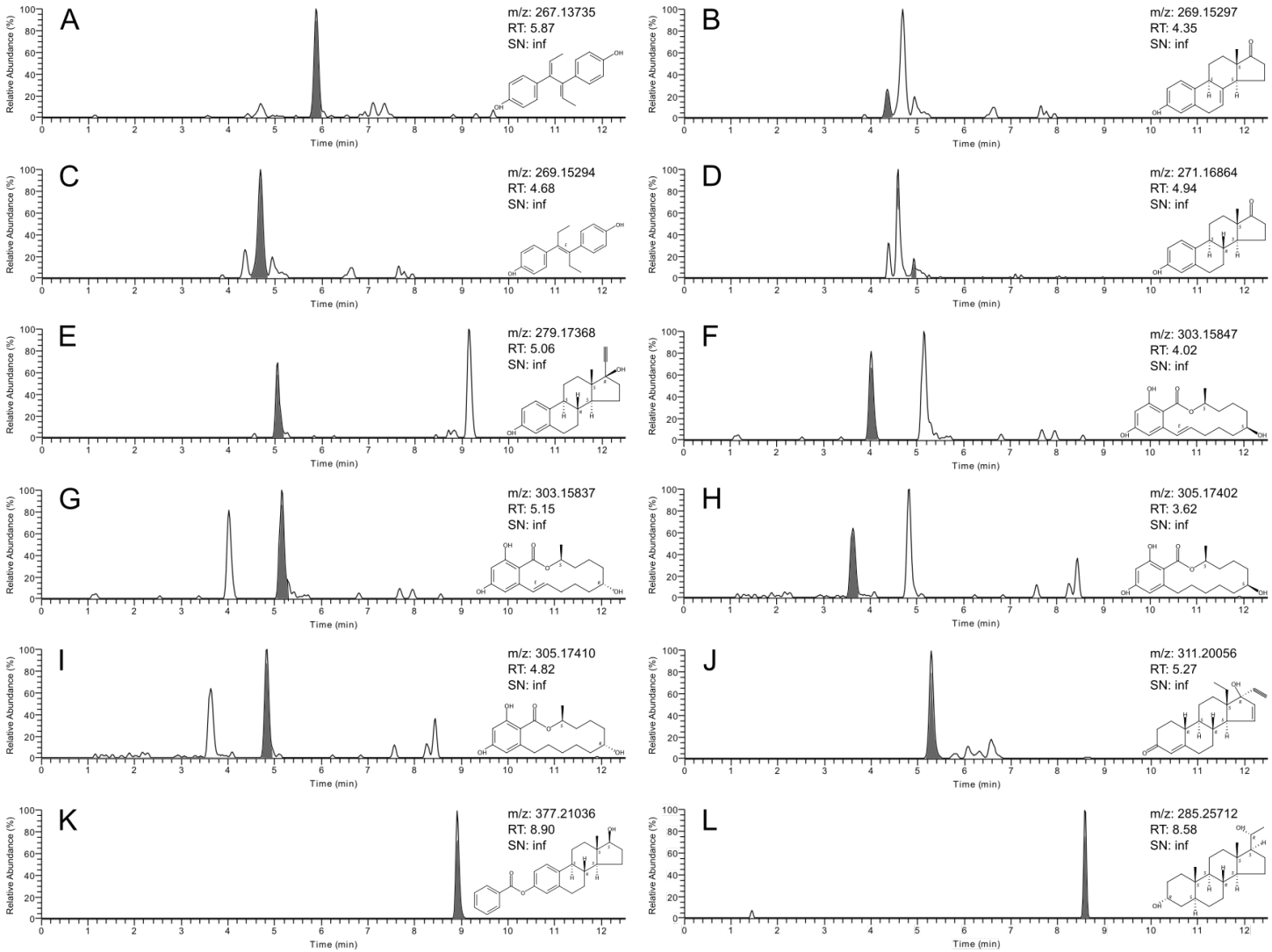


Figure A4. UHPLC-HR-Q-Orbitrap-MS chromatograms of the oestrogens included in this method (part II), i.e. (a) dienoestrol, (b) equilin, (c) diethylstilbestrol, (d) estrone, (e) 17α-ethinylestradio, (f) β-zearalenol, (g) α-zearalenol, (h) β-zeranol, (i) α-zeranol, (j) gestodene, (k) estradiol-benzoate, and progestins, i.e. (l) 5α-pregnan-3α,20β-diol.

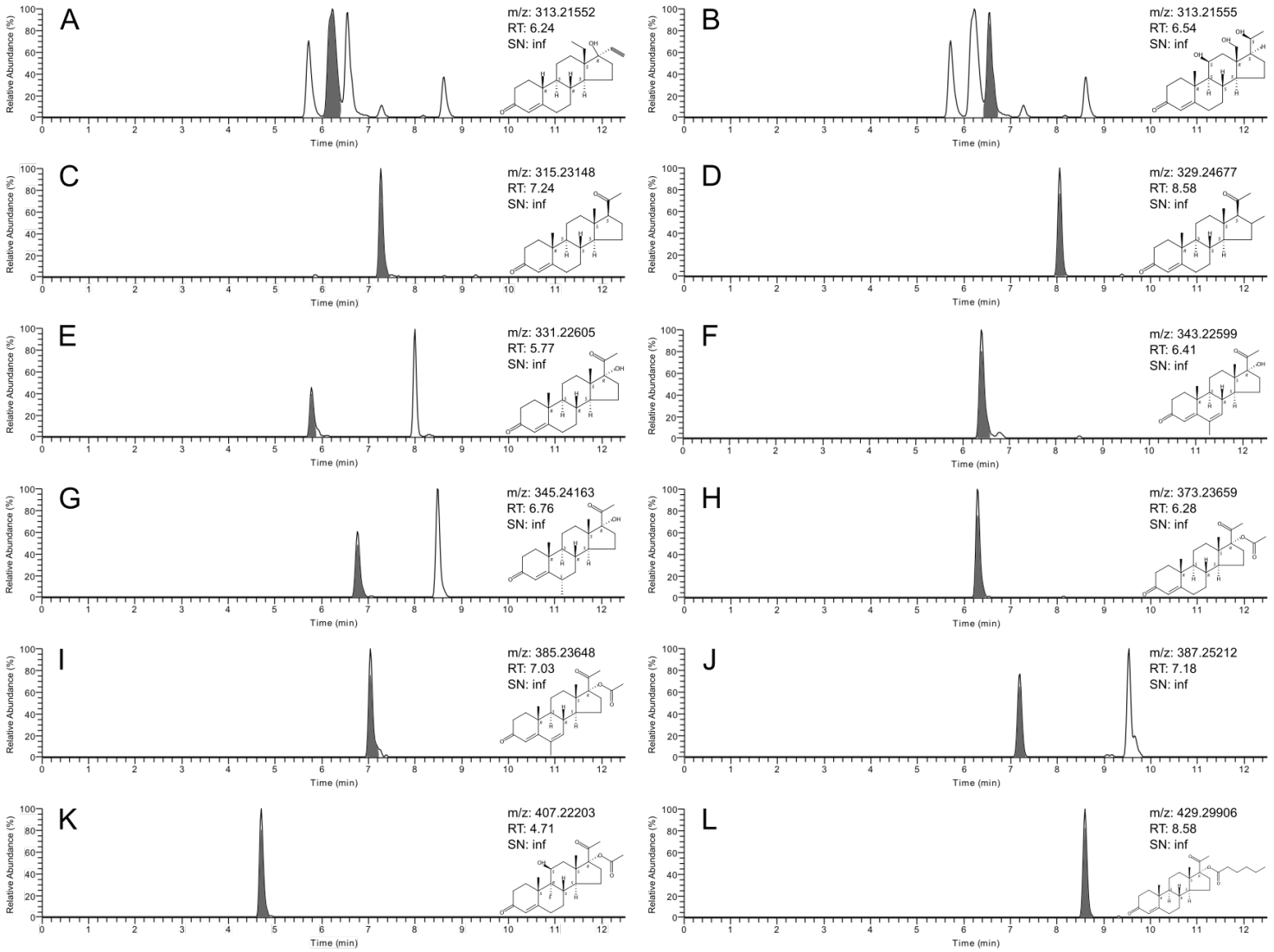
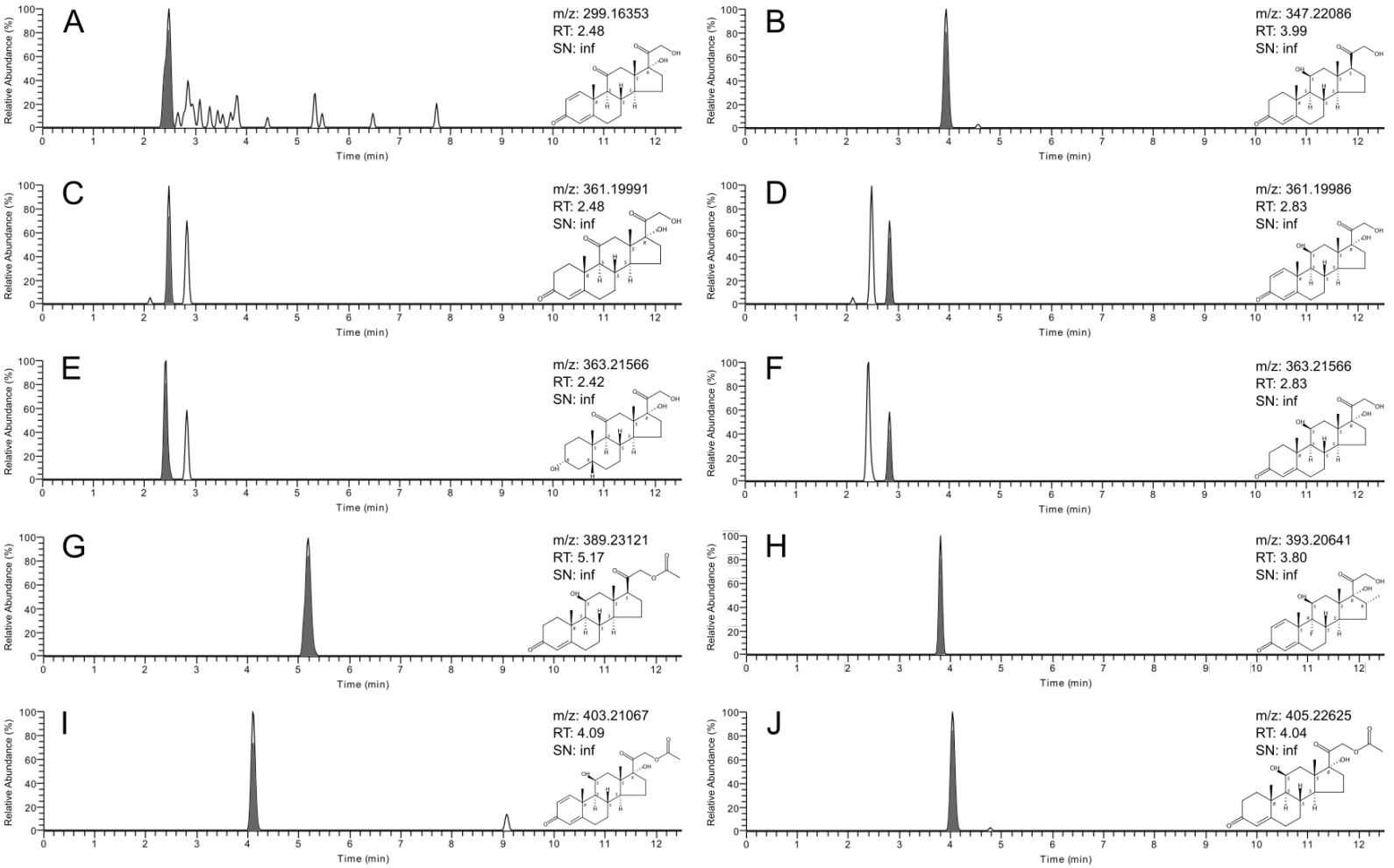


Figure A5. UHPLC-HR-Q-Orbitrap-MS chromatograms of the progestins included in this method, i.e. (a) norgestrel, (b) dihydroprogesterone, (c) progesterone, (d) methylprogesterone, (e) 17α-hydroxyprogesterone, (f) megestrol, (g) medroxyprogesterone, (h)17α-acetoxyprogesterone, (i) megestrol acetate, (j) medroxyprogesterone acetate, (k) flugestone acetate and (l) caproxyprogesterone.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D
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RT: 2.83
SN: inf
E
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RT: 2.42
SN: inf
F
m/z: 363.21566
RT: 2.83
SN: inf
G
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RT: 5.17
SN: inf
H
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RT: 3.80
SN: inf
I
m/z: 403.21067
RT: 4.09
SN: inf
J
m/z: 405.22625
RT: 4.04
SN: inf
Relative Abundance (%)
Time (min)

Table A1. The target EDC compounds including CAS number, molecular mass (g mol^{-1}), pKa, log P, water solubility (mg L^{-1}), vapor pressure (Torr) and bioconcentration factor (L kg^{-1}) at a temperature of 25°C and pH 7.

Analyte	CAS number	Molecular mass (g mol^{-1})	pKa	Log P	Water solubility (mg L^{-1})	Vapor Pressure (Torr)	Bioconcentration factor (L kg^{-1})
Androgens							
Methandriol	521-10-8	304.47	15.01 ± 0.70	4.328 ± 0.303	4.60E+00	3.25E-09	1.15E+03
17α-trenbolone	80657-17-6	270.37	14.73 ± 0.40	2.316 ± 0.350	1.20E+01	1.09E-11	1.51E+02
17β-trenbolone	10161-33-8	270.37	14.73 ± 0.40	3.169 ± 0.401	1.20E+01	1.09E-11	1.51E+02
11β-hydroxyandrosterone	57-61-4	306.44	14.62 ± 0.70	1.972 ± 0.333	1.20E+02	3.51E-10	1.86E+01
Testosterone 17β-cypionate	58-20-8	412.60	-	6.608 ± 0.295	4.50E-02	3.78E-11	6.20E+04
17β-dihydroandrosterone	1852-53-5	292.46	15.07 ± 0.70	4.394 ± 0.268	4.10E+00	1.27E-08	1.29E+03
Androsterone	53-41-8	290.44	15.15 ± 0.60	3.932 ± 0.325	7.30E+00	1.50E-08	5.74E+02
19-nortestosterone	434-22-0	274.40	15.06 ± 0.40	2.898 ± 0.263	2.40E+01	2.25E-09	9.38E+01
1,4-Androstadienedione	897-06-3	284.39	-	2.623 ± 0.412	3.40E+01	9.62E-08	5.80E+01
11-ketoetiocholanolone	739-27-5	304.42	15.07 ± 0.60	1.894 ± 0.398	1.10E+02	3.97E-10	1.62E+01
Androstenedione	63-05-8	286.41	-	2.717 ± 0.340	5.78E+01	1.20E-07	6.84E+01
Mestanolone	521-11-9	304.47	15.15 ± 0.60	4.313 ± 0.351	4.90E+00	1.16E-08	1.12E+03
17α-testosterone	481-30-1	288.42	15.06 ± 0.60	3.179 ± 0.277	2.00E+01	2.60E-09	1.53E+02
17β-testosterone	58-22-0	288.42	15.06 ± 0.60	3.179 ± 0.277	2.00E+01	2.60E-09	1.53E+02
5α-dihydrotestosterone	521-18-6	290.44	15.08 ± 0.60	3.932 ± 0.325	7.30E+00	1.50E-08	5.74E+02
19-Norethindron	68-22-4	298.42	13.09 ± 0.40	2.858 ± 0.320	3.00E+01	7.22E-10	8.76E+01
Methylboldenone	72-63-9	300.44	15.12 ± 0.60	3.465 ± 0.382	1.40E+01	1.87E-09	2.53E+02
11-ketotestosterone	564-35-2	302.41	14.79 ± 0.60	1.296 ± 0.470	2.50E+02	4.30E-11	5.69E+00
Formestane	566-48-3	302.41	9.31 ± 0.60	1.785 ± 0.342	1.20E+02	4.91E-11	1.34E+01
Norethandrolone	52-78-8	302.45	15.13 ± 0.40	3.778 ± 0.278	9.40E+00	7.11E-10	4.45E+02
Methyltestosterone	58-18-4	302.45	15.13 ± 0.60	3.559 ± 0.303	1.40E+01	2.28E-09	2.98E+02
Trenbolone acetate	10161-34-9	312.40	-	4.019 ± 0.407	3.10E+00	1.26E-09	6.67E+02
Ethynyl testosterone	434-03-7	312.45	13.10 ± 0.60	3.139 ± 0.349	2.40E+01	7.59E-10	1.43E+02

Analyte	CAS number	Molecular mass (g mol^{-1})	pKa	Log P	Water solubility (mg L^{-1})	Vapor Pressure (Torr)	Bioconcentration factor (L kg^{-1})
Stanozolol	10418-03-8	328.49	15.15 ± 0.60	5.410 ± 0.330	1.10E+00	1.89E-10	7.61E+03
Testosterone acetate	434-03-7	330.46	13.10 ± 0.60	3.139 ± 0.349	2.40E+01	7.59E-10	1.43E+02
Fluoxymesterone	76-43-7	336.44	13.40 ± 0.70	2.269 ± 0.404	4.40E+01	5.50E-11	3.12E+01
Testosterone propionate	911657-75-5	402.52	-	4.392 ± 0.334	1.30E+00	6.27E-11	1.28E+03
Chlorotestosteron acetate	855-19-6	364.91	-	4.606 ± 0.298	1.00E+00	1.52E-08	1.86E+03
Testosterone benzoate	42723-70-6	392.53	-	5.993 ± 0.292	9.00E-02	5.20E-11	2.11E+04
Testosterone phenylpropionate	1255-49-8	420.58	-	6.286 ± 0.298	5.00E-02	5.16E-12	3.52E+04
19-nortestosterone-17-decanoate	360-70-3	428.65	-	7.939 ± 0.273	1.95E-02	1.30E-11	6.36E+05
Oestrogens							
17α-estradiol	57-91-0	272.38	10.27 ± 0.60	4.146 ± 0.250	3.00E+00	9.82E-09	8.33E+02
17β-estradiol	50-28-2	272.38	10.27 ± 0.60	4.146 ± 0.256	3.00E+00	9.82E-09	8.33E+02
Estradiol-17-acetate	1743-60-8	314.42	10.26 ± 0.60	5.111 ± 0.267	6.90E-01	9.88E-09	4.51E+03
Dienoestrol	84-17-3	266.33	9.21 ± 0.15	4.920 ± 0.309	5.60E+00	8.07E-07	3.23E+03
Equilin	474-86-2	268.35	10.11 ± 0.40	2.271 ± 0.414	2.00E+01	4.76E-09	6.89E+01
Diethylstilbestrol	56-53-1	268.29	10.18 ± 0.26	5.330 ± 0.300	6.70E+00	3.29E-07	6.62E+03
Estrone	53-16-7	270.37	10.25 ± 0.40	3.624 ± 0.369	5.70E+00	1.54E-08	3.35E+02
17α-ethinylestradiol	57-63-6	296.40	10.24 ±0.60	4.106 ± 0.315	3.90E+00	3.74E-09	7.78E+02
α-zearalenol	36455-72-8	320.38	7.61 ± 0.60	4.168 ± 0.790	1.90E+03	3.40E-15	1.14E+02
β-zearalenol	71030-11-0	320.38	7.61 ± 0.60	4.168 ± 0.790	1.90E+03	3.40E-15	1.14E+02
α-zeranol	26538-44-3	322.40	8.08 ± 0.60	3.085 ± 0.359	5.50E+02	4.16E-14	1.74E+03
β-zeranol	42422-68-4	322.40	8.08 ± 0.60	3.085 ± 0.359	5.50E+02	4.16E-14	1.74E+03
Gestodene	60282-87-3	310.43	12.16 ± 0.40	2.022 ± 0.436	9.00E-01	1.66E-10	2.03E+01
Estradiol-benzoate	50-50-0	376.49	15.06 ± 0.40	5.095 ± 0.360	1.50E-01	4.08E-12	4.39E+03
Progestins							
5α-Pregnan-3α,20β-diol	21152-50-1	480.64	-3.50 ± 0.18	5.448 ± 0.492	1.80E+03	1.00E+00	-

Analyte	CAS number	Molecular mass (g mol^{-1})	pKa	Log P	Water solubility (mg L^{-1})	Vapor Pressure (Torr)	Bioconcentration factor (L kg^{-1})
Norgestrel	797-63-7	312.45	13.09 ± 0.40	3.368 ± 0.325	1.70E+01	2.32E-10	2.14E+02
Dihydroprogesterone	165036-75-9	348.48	14.54 ± 0.70	0.561 ± 0.301	9.40E+02	7.65E-14	1.57E+00
Progesterone	57-83-0	314.60	-	3.827 ± 0.282	9.40E+00	3.44E-08	4.47E+02
Methylprogesterone	896438-14-5	450.57	3.09 ± 0.70	5.209 ± 0.605	5.90E+02	5.05E-16	1.62E+00
17α-hydroxyprogesterone	68-96-2	330.46	13.03 ± 0.60	3.040 ± 0.362	2.40E+01	2.37E-11	1.20E+02
Megestrol	3562-63-8	342.47	13.00 ± 0.70	3.225 ± 0.410	1.80E+01	4.15E-12	1.66E+02
Medroxyprogesterone	520-85-4	344.49	13.03 ± 0.70	3.576 ± 0.365	1.20E+01	1.43E-11	3.07E+02
17α-acetoxyprogesterone	302-23-8	372.50	-	3.638 ± 0.435	7.80E+00	9.34E-10	3.43E+02
Megestrol acetate	595-33-5	384.51	-	3.748 ± 0.465	6.50E+00	2.10E-10	4.16E+02
Medroxyprogesterone acetate	71-58-9	344.49	-	4.174 ± 0.438	3.70E+00	5.44E-10	8.75E+02
Flugestone acetate	2529-45-5	406.49	13.09 ± 0.70	2.816 ± 0.494	1.10E+01	2.72E-13	8.13E+01
Caproxyprogesterone	630-56-8	428.60	-	5.676 ± 0.435	4.10E-01	1.40E-11	1.21E+04
Corticosteroids							
Prednisone	53-03-2	358.43	12.36 ± 0.60	1.566 ± 0.793	1.00E+02	1.51E-15	9.12E+00
Corticosterone	50-22-6	346.46	12.98 ± 0.10	1.952 ± 0.399	9.70E+01	2.07E-13	1.79E+01
Cortisone	53-06-5	360.44	12.37 ± 0.60	1.433 ± 0.662	1.40E+02	2.96E-15	7.23E+00
Prednisolone	50-24-8	360.44	12.47 ± 0.70	1.635 ± 0.526	1.00E+02	2.13E-15	1.03E+01
Cortisol	50-23-7	362.46	12.47 ± 0.70	1.762 ± 0.471	9.40E+01	3.44E-15	1.29E+01
Tetrahydrocortisone	53-05-4	364.48	12.38 ± 0.70	2.109 ± 0.568	6.20E+01	4.00E-14	2.36E+01
Corticosterone acetate	1173-26-8	388.50	14.48 ± 0.70	2.308 ± 0.464	4.70E+01	5.14E-14	3.35E+01
Dexamethasone	50-02-2	392.46	12.13 ± 0.70	2.033 ± 0.573	3.50E+01	2.81E-15	2.07E+01
Prednisolone acetate	52-21-1	402.48	12.41 ± 0.70	2.256 ± 0.581	3.10E+01	7.36E-16	3.05E+01
Cortisone acetate	50-04-4	402.48	12.32 ± 0.60	2.054 ± 0.707	4.40E+01	9.94E-16	2.14E+01
Hydrocortisone 21-acetate	50-03-3	404.50	12.42 ± 0.70	2.383 ± 0.533	2.80E+01	1.08E-15	3.81E+01

Table A2. Screening parameters with their specific ranges for the optimization of the extraction of EDCs from seawater. Parameters are listed in the order that they occur during execution of the extraction procedure.

Parameters	Type	Unit	Ranges		
			Lower level (-1)	Central level (0)	Upper level (+1)
Pretreatment	Categorical	/	Whatman filter	/	No filter
pH adjustment sample	Continuous	/	3.0	5.5	8.0
SPE-cartridge	Categorical	/	H_2O phillic DVB Speedisk	/	C_{18} Speedisk
Volume conditioning	Continuous	mL	20	/	40
Volume equilibration	Continuous	mL	20	/	40
Loading volume	Continuous	mL	1000	/	2500
Wash volume	Continuous	mL	20	/	40
Dry time	Continuous	min	5	/	20
Elution solvent	Categorical	/	MeCN	/	MeOH
Solvent additive	Categorical	/	0.1% FA	Absent	0.1% NH_4OH
Elution volume	Continuous	mL	5	/	10
Evaporation temperature	Continuous	°C	30	/	50
Centrifuging	Categorical	αg	0	/	2430

Table A3. Detailed results of the method validation for EDCs in seawater.

Analyte	Nominal concentration (ng L^{-1})	Recovery (%)	MDL (ng L^{-1})	MQL (ng L^{-1})	Repeatability RSD (%) (n = 18)	Within-laboratory reproducibility RSD (%) (n = 12)	Linearity R^2
Androgens							
Methandriol	0.75	103.1 ± 9.8	0.06	0.50	9.5	3.8	0.9980
	1.00	101.1 ± 5.7			5.6	5.6	
	1.25	99.8 ± 3.5			3.5	1.9	
17β-trenbolone	0.75	100.9 ± 7.0	0.25	0.50	7.0	9.6	0.9993
	1.00	98.6 ± 5.4			5.5	6.8	
	1.25	100.5 ± 4.0			4.0	4.0	
17α-trenbolone	0.75	102.8 ± 13.4	0.25	0.50	7.0	9.6	0.9949
	1.00	100.3 ± 4.8			5.5	6.8	
	1.25	101.6 ± 4.6			4.0	4.0	
11β-hydroxyandrosterone	0.75	102.8 ± 13.4	0.25	0.50	13.1	14.3	0.9949
	1.00	100.3 ± 4.8			4.8	4.8	
	1.25	101.6 ± 4.6			4.5	4.5	
Testosterone 17β-cypionate	0.75	109.9 ± 7.8	0.13	0.50	7.1	11.8	0.9981
	1.00	107.3 ± 5.3			4.9	8.9	
	1.25	107.6 ± 5.7			5.3	5.4	
17β-dihydroandrosterone	0.75	95.3 ± 6.8	0.13	0.50	7.0	7.3	0.9903
	1.00	97.2 ± 6.4			6.2	6.7	
	1.25	98.9 ± 5.3			5.5	6.0	
Androsterone	0.38	103.7 ± 5.4	0.25	0.25	5.2	5.5	0.9968
	0.50	99.0 ± 3.1			3.1	4.7	
	0.63	99.7 ± 2.7			2.7	2.8	
19-nortestosterone	1.13	96.4 ± 5.7	0.13	0.75	5.9	7.8	0.9984

Analyte	Nominal concentration ($ng\ L^{-1}$)	Recovery (%)	MDL ($ng\ L^{-1}$)	MQL ($ng\ L^{-1}$)	Repeatability RSD (%) (n = 18)	Within-laboratory reproducibility RSD (%) (n = 12)	Linearity R^2
	1.50	98.1 ± 4.8			4.9	7.7	
	1.88	97.6 ± 3.6			3.7	3.4	
1,4-Androstadienedione	0.38	95.0 ± 8.0	0.06	0.25	8.4	13.2	0.9987
	0.50	98.2 ± 8.0			8.1	5.0	
	0.63	99.1 ± 7.1			7.2	3.1	
11-ketoetiocholanolone	0.38	97.9 ± 10.8	0.13	0.25	11.0	13.6	0.9985
	0.50	97.6 ± 6.8			7.0	7.2	
	0.63	99.3 ± 3.0			3.0	3.0	
Androstenedione	0.75	94.3 ± 4.0	0.13	0.50	4.3	6.6	0.9995
	1.00	99.5 ± 5.8			5.8	5.1	
	1.25	98.4 ± 2.8			2.8	5.3	
Mestanolone	1.13	99.9 ± 5.8	0.25	0.75	5.8	7.9	0.9965
	1.50	98.8 ± 4.7			4.8	6.5	
	1.88	100.7 ± 4.3			4.3	5.4	
17β-testosterone	0.38	96.8 ± 7.5	0.13	0.25	7.7	7.7	0.9975
	0.50	101.3 ± 4.6			4.5	7.0	
	0.63	102.9 ± 4.2			4.1	4.1	
17α-testosterone	0.38	97.0 ± 8.4	0.06	0.25	8.7	14.4	0.9998
	0.50	98.0 ± 6.3			5.8	7.9	
	0.63	101.7 ± 4.6			2.8	6.5	
5α-dihydrotestosterone	0.38	96.8 ± 6.9	0.13	0.25	7.1	7.3	0.9923
	0.50	98.3 ± 6.3			6.1	6.4	
	0.63	99.4 ± 5.8			5.9	6.0	
19-Norethindron	1.50	95.4 ± 7.2	0.50	1.00	7.6	10.1	0.9975

Analyte	Nominal concentration (ng L^{-1})	Recovery (%)	MDL (ng L^{-1})	MQL (ng L^{-1})	Repeatability RSD (%) (n = 18)	Within-laboratory reproducibility RSD (%) (n = 12)	Linearity R^2
	2.00	98.9 ± 3.1			3.1	6.6	
	2.50	100.1 ± 4.4			4.4	4.9	
Methylboldenone	1.50	99.2 ± 9.2	0.25	1.00	9.3	10.1	0.9985
	2.00	101.3 ± 7.2			7.1	8.2	
	2.50	100.9 ± 4.3			4.3	4.9	
11-ketotestosterone	0.38	97.9 ± 8.3	0.13	0.25	8.5	8.9	0.9974
	0.50	101.3 ± 7.2			7.1	8.5	
	0.63	99.5 ± 2.9			2.9	3.4	
Formestane	0.38	99.3 ± 9.5	0.13	0.25	9.5	8.3	0.9965
	0.50	100.2 ± 8.0			8.0	12.0	
	0.63	102.4 ± 6.2			6.0	5.9	
Norethandrolone	0.19	104.7 ± 5.9	0.06	0.13	5.6	8.7	0.9962
	0.25	100.4 ± 8.0			8.0	7.9	
	0.31	100.4 ± 3.7			3.7	3.7	
Methyltestosterone	0.38	97.6 ± 5.9	0.13	0.25	6.1	8.4	0.9992
	0.50	98.1 ± 4.4			4.5	5.7	
	0.63	100.6 ± 3.8			3.8	3.9	
Trenbolone acetate	0.75	103.4 ± 6.2	0.06	0.50	6.0	9.5	0.9951
	1.00	100.5 ± 4.2			4.2	4.4	
	1.25	97.8 ± 3.7			3.8	3.9	
Ethynyl testosterone	0.38	106.9 ± 8.5	0.06	0.25	7.9	9.1	0.9987
	0.50	102.3 ± 6.0			5.9	6.0	
	0.63	100.7 ± 2.6			2.6	2.8	
Stanozolol	1.50	99.1 ± 8.0	1.00	1.00	8.0	13.9	0.9977

Analyte	Nominal concentration ($ng\ L^{-1}$)	Recovery (%)	MDL ($ng\ L^{-1}$)	MQL ($ng\ L^{-1}$)	Repeatability RSD (%) (n = 18)	Within-laboratory reproducibility RSD (%) (n = 12)	Linearity R^2
	2.00	97.1 ± 5.1			5.3	7.5	
	2.50	100.5 ± 2.5			2.5	2.5	
Testosterone acetate	1.13	100.3 ± 6.4	0.06	0.75	6.3	10.0	0.9983
	1.50	99.7 ± 3.8			3.8	8.3	
	1.88	101.7 ± 3.9			3.9	4.5	
Fluoxymesterone	7.50	102.8 ± 7.5	2.50	5.00	7.3	7.4	0.9975
	10.00	102.3 ± 3.6			3.6	7.1	
	12.50	100.8 ± 4.0			4.0	5.0	
Testosterone propionate	0.38	98.8 ± 6.6	0.13	0.25	6.7	9.0	0.9973
	0.50	102.3 ± 5.7			5.5	8.0	
	0.63	100.0 ± 3.2			3.2	4.5	
Chlorotestosteron acetate	0.75	98.9 ± 4.1	0.50	0.50	4.2	9.5	0.9962
	1.00	102.7 ± 4.5			4.4	8.2	
	1.25	100.1 ± 3.1			3.1	3.7	
Testosterone benzoate	1.13	104.6 ± 7.1	0.50	0.75	6.8	11.5	0.9978
	1.50	101.8 ± 4.9			4.8	8.3	
	1.88	102.2 ± 3.2			3.1	5.4	
Testosterone phenylpropionate	1.13	100.8 ± 7.9	0.25	0.75	7.8	8.7	0.9952
	1.50	100.7 ± 4.9			4.9	8.0	
	1.88	100.8 ± 3.7			3.6	4.4	
19-nortestosterone-17-decanoate	3.75	102.3 ± 7.2	2.50	2.50	7.0	9.3	0.9926
	5.00	102.6 ± 6.1			6.0	8.5	
	6.25	102.7 ± 3.4			3.3	5.0	

Analyte	Nominal concentration (ng L^{-1})	Recovery (%)	MDL (ng L^{-1})	MQL (ng L^{-1})	Repeatability RSD (%) (n = 18)	Within-laboratory reproducibility RSD (%) (n = 12)	Linearity R^2
Oestrogens/Estranes							
17β-estradiol	7.50	100.9 ± 8.5	0.25	5.00	8.4	9.0	0.9976
	10.00	102.2 ± 9.3			9.1	8.7	
	12.50	101.7 ± 3.6			3.5	3.5	
17α-estradiol	3.75	100.0 ± 10.2	0.06	2.50	10.2	14.0	0.9959
	5.00	100.8 ± 5.7			5.7	7.4	
	6.25	101.8 ± 5.3			5.2	5.2	
Estradiol-17-acetate	1.13	98.9 ± 10.7	0.06	0.75	10.8	14.1	0.9937
	1.50	101.5 ± 5.1			5.1	5.5	
	1.88	100.8 ± 4.2			4.1	4.5	
Dienoestrol	7.50	99.6 ± 8.8	0.25	5.00	8.8	13.0	0.9964
	10.00	99.0 ± 7.0			7.1	9.2	
	12.50	102.3 ± 3.1			3.1	3.0	
Equilin	0.38	105.1 ± 9.7	0.13	0.25	9.3	10.5	0.9950
	0.50	99.3 ± 6.8			6.9	10.1	
	0.63	101.5 ± 3.1			3.0	2.9	
Diethylstilbestrol	0.38	102.4 ± 9.6	0.25	0.25	9.3	13.3	0.9958
	0.50	102.2 ± 5.5			5.4	13.4	
	0.63	100.8 ± 4.0			4.0	3.4	
Estrone	0.38	103.0 ± 8.7	0.06	0.25	8.4	11.8	0.9992
	0.50	102.5 ± 6.8			6.7	8.5	
	0.63	102.7 ± 5.0			4.9	5.4	
17α-ethinylestradiol	7.50	102.5 ± 6.6	2.50	5.00	6.5	11.8	0.9958

Analyte	Nominal concentration (ng L^{-1})	Recovery (%)	MDL (ng L^{-1})	MQL (ng L^{-1})	Repeatability RSD (%) (n = 18)	Within-laboratory reproducibility RSD (%) (n = 12)	Linearity R^2
	10.00	102.7 ± 4.9			4.8	8.0	
	12.50	103.1 ± 2.9			2.8	3.5	
β-zearalenol	3.75	106.3 ± 9.4	1.00	2.50	8.9	12.6	0.9921
	5.00	101.6 ± 7.2			7.1	7.4	
	6.25	95.4 ± 2.4			3.6	4.1	0.9931
α-zearalenol	1.13	101.6 ± 8.9	0.13	0.75	8.8	11.0	
	1.50	101.9 ± 6.0			5.9	8.2	
	1.88	100.7 ± 3.2			3.2	3.6	
β-zeranol	1.13	98.1 ± 9.4	0.13	0.75	9.5	14.0	0.9947
	1.50	99.9 ± 9.7			9.7	8.5	
	1.88	101.8 ± 5.3			5.2	6.5	
α-zeranol	1.13	101.8 ± 8.6	0.13	0.75	8.5	9.1	0.9908
	1.50	101.1 ± 6.6			6.5	7.7	
	1.88	101.4 ± 5.0			4.9	4.2	
Gestodene	0.75	106.6 ± 7.1	0.25	0.50	6.7	9.8	0.9946
	1.00	101.2 ± 5.6			5.5	8.0	
	1.25	102.3 ± 3.1			3.0	3.1	
Estradiol-benzoate	3.75	103.5 ± 5.4	1.00	2.50	5.2	8.7	0.9941
	5.00	100.2 ± 5.1			5.1	7.6	
	6.25	99.9 ± 4.4			4.4	4.4	
Progestins							
5α-Pregnan-3α,20β-diol	3.75	101.1 ± 5.6	2.50	2.50	5.2	7.2	0.9917
	5.00	101.8 ± 4.2			3.9	5.2	
	6.25	101.0 ± 3.2			2.9	4.9	

Analyte	Nominal concentration (ng L^{-1})	Recovery (%)	MDL (ng L^{-1})	MQL (ng L^{-1})	Repeatability RSD (%) (n = 18)	Within-laboratory reproducibility RSD (%) (n = 12)	Linearity R^2
Norgestrel	0.38	100.8 ± 6.5	0.06	0.25	6.5	8.0	0.9949
	0.50	102.4 ± 4.9			4.8	6.6	
	0.63	99.9 ± 4.6			4.6	5.7	
Dihydroprogesterone	0.38	96.5 ± 7.1	0.06	0.25	7.4	10.0	0.9973
	0.50	98.6 ± 6.5			6.6	8.3	
	0.63	99.7 ± 5.3			5.3	6.0	
Progesterone	0.75	93.7 ± 11.0	0.06	0.50	11.7	14.0	0.9984
	1.00	102.3 ± 5.4			5.3	8.4	
	1.25	101.0 ± 3.2			3.2	4.0	
Methylprogesterone	0.38	103.2 ±7.5	0.06	0.25	7.3	8.1	0.9961
	0.50	103.0 ± 5.4			5.2	5.7	
	0.63	100.4 ± 3.9			3.9	4.0	
17α-hydroxyprogesterone	0.38	96.8 ± 8.8	0.13	0.25	9.1	11.0	0.9986
	0.50	102.6 ± 4.9			4.8	4.4	
	0.63	100.9 ± 3.1			3.1	3.6	
Megestrol	1.50	101.0 ± 8.0	0.75	1.00	7.9	12.1	0.9966
	2.00	105.4 ± 5.7			5.4	8.5	
	2.50	99.4 ± 3.7			3.7	5.7	
Medroxyprogesterone	0.75	101.7 ± 4.8	0.13	0.50	4.8	5.9	0.9966
	1.00	100.7 ± 3.8			3.8	4.4	
	1.25	101.1 ± 3.6			3.6	3.7	
17α-acetoxyprogesterone	0.75	103.3 ± 4.4	0.13	0.50	4.3	8.5	0.9952
	1.00	103.4 ± 4.5			4.4	7.4	
	1.25	100.9 ± 3.4			3.4	3.4	

Analyte	Nominal concentration ($ng\ L^{-1}$)	Recovery (%)	MDL ($ng\ L^{-1}$)	MQL ($ng\ L^{-1}$)	Repeatability RSD (%) (n = 18)	Within-laboratory reproducibility RSD (%) (n = 12)	Linearity R^2
Megestrol acetate	1.13	101.3 ± 5.8	0.50	0.75	5.7	5.5	0.9976
	1.50	102.2 ± 4.3			4.2	5.5	
	1.88	101.7 ± 3.4			3.4	3.2	
Medroxyprogesterone acetate	1.50	102.1 ± 3.9	0.50	1.00	3.8	4.4	0.9976
	2.00	101.7 ± 3.8			3.7	4.3	
	2.50	100.6 ± 3.0			3.0	3.2	
Flugestone acetate	1.50	101.1 ± 7.5	0.75	1.00	7.4	8.1	0.9969
	2.00	105.5 ± 8.6			8.0	8.9	
	2.50	101.0 ± 3.3			3.2	3.1	
Caproxyprogesterone	1.13	102.0 ± 4.7	0.25	0.75	4.6	10.8	0.9977
	1.50	102.0 ± 4.4			4.3	8.0	
	1.88	101.9 ± 4.4			4.3	6.6	
Corticosteroids							
Prednisone	0.75	104.6 ± 8.2	0.25	0.50	7.8	10.1	0.9914
	1.00	104.3 ± 6.4			6.1	6.7	
	1.25	101.4 ± 4.2			4.2	7.0	
Corticosterone	3.75	100.3 ± 8.0	0.50	2.50	8.0	10.8	0.9909
	5.00	104.1 ± 6.5			6.2	6.7	
	6.25	101.7 ± 3.4			3.3	4.8	
Cortisone	3.75	95.9 ± 11.4	0.13	2.50	11.9	13.6	0.9952
	5.00	106.6 ± 6.3			5.9	10.6	
	6.25	102.7 ± 4.9			4.8	7.3	
Prednisolone	3.75	103.5 ± 9.8	0.13	2.50	9.5	9.6	0.9985

Analyte	Nominal concentration ($ng\ L^{-1}$)	Recovery (%)	MDL ($ng\ L^{-1}$)	MQL ($ng\ L^{-1}$)	Repeatability RSD (%) (n = 18)	Within-laboratory reproducibility RSD (%) (n = 12)	Linearity R^2
	5.00	103.7 ± 7.7			7.4	8.5	
	6.25	101.2 ± 4.3			4.2	6.4	
Cortisol	1.13	99.7 ± 13.6	0.25	0.75	13.7	14.3	0.9926
	1.50	104.6 ± 7.6			7.3	8.8	
	1.88	102.7 ± 4.0			3.9	6.8	
Tetrahydrocortisone	7.50	102.0 ± 9.2	0.25	5.00	9.0	11.6	0.9985
	10.00	101.2 ± 5.3			5.2	10.4	
	12.50	104.3 ± 5.5			5.3	5.8	
Corticosterone acetate	3.75	106.6 ± 6.1	0.50	2.50	5.7	8.4	0.9952
	5.00	101.0 ± 4.2			4.2	4.6	
	6.25	101.3 ± 2.8			2.8	3.5	
Dexamethasone	3.75	106.6 ± 6.1	2.50	2.50	5.7	8.4	0.9952
	5.00	101.0 ± 4.2			4.2	4.6	
	6.25	101.3 ± 2.8			2.8	3.5	
Prednisolone acetate	5.00	97.5 ± 5.2	5.00	5.00	5.3	8.5	0.9906
	10.00	101.5 ± 7.5			7.4	8.0	
	15.00	100.7 ± 4.8			4.8	4.8	
Cortisone acetate	3.75	101.0 ± 11.5	2.50	2.50	11.3	11.4	0.9952
	5.00	102.6 ± 6.6			6.4	7.4	
	6.25	100.4 ± 5.6			5.6	3.9	
Hydrocortisone 21-acetate	5.00	99.1 ± 8.3	5.00	5.00	8.3	13.7	0.9984
	10.00	99.6 ± 7.0			7.0	8.1	
	15.00	101.3 ± 4.8			4.7	4.2	

Table A4. Calibration equations for the target EDCs in sea and fresh water, obtained by weighted linear regression.

Analyte	Seawater								Fresh water							
	Equation						Dynamic range (ng L^{-1})		Equation						Dynamic range (ng L^{-1})	
Androgens																
Methandriol	y =	-1.14E-01	+	8.62E-02	x		0.00 - 50.00		y =	2.96E-02	+	5.67E-02	x		0.00 - 50.00	
17α-trenbolone	y =	-8.45E-02	+	1.87E-01	x		0.00 - 50.00		y =	-3.73E-02	+	1.88E-01	x		0.00 - 40.00	
17β-trenbolone	y =	-1.85E-02	+	7.05E-02	x		0.00 - 50.00		y =	2.96E-02	+	5.67E-02	x		0.00 - 40.00	
11β-hydroxyandrosterone	y =	-6.99E-02	+	1.22E-01	x		0.00 - 40.00		y =	-4.44E-02	+	6.79E-02	x		0.00 - 40.00	
Testosterone 17β–cypionate	y =	-6.29E-02	+	1.41E-01	x		0.00 - 40.00		y =	-2.79E-02	+	6.19E-02	x		0.00 - 40.00	
17β-dihydroandrosterone	y =	-2.08E-02	+	1.66E-01	x		0.00 - 50.00		y =	-5.31E-02	+	1.43E-01	x		0.00 - 40.00	
Androsterone	y =	8.06E-02	+	1.08E-01	x		0.00 - 50.00		y =	-4.27E-02	+	1.03E-01	x		0.00 - 40.00	
19-nortestosterone	y =	-1.78E-01	+	2.00E-01	x		0.00 - 50.00		y =	-6.35E-02	+	1.86E-01	x		0.00 - 50.00	
1,4-Androstadienedione	y =	-7.73E-02	+	1.49E-01	x		0.00 - 50.00		y =	-1.21E-01	+	1.29E-01	x		0.00 - 40.00	
11-ketoetiocholanolone	y =	-1.67E-02	+	1.41E-01	x		0.00 - 50.00		y =	-6.99E-02	+	1.57E-01	x		0.00 - 40.00	
Androstenedione	y =	-2.50E-02	+	1.91E-01	x		0.00 - 50.00		y =	3.39E-02	+	1.67E-01	x		0.00 - 40.00	
Mestanolone	y =	1.72E-01	+	1.28E-01	x		0.00 - 50.00		y =	1.43E-01	+	9.73E-02	x		0.00 - 40.00	
17α-testosterone	y =	-8.33E-02	+	3.49E-01	x		0.00 - 50.00		y =	-7.84E-03	+	2.83E-01	x		0.00 - 50.00	
17β-testosterone	y =	2.55E-01	+	2.63E-01	x		0.00 - 40.00		y =	-2.50E-02	+	3.01E-01	x		0.00 - 40.00	
5α-dihydrotestosterone	y =	-2.28E-01	+	1.35E-01	x		0.00 - 50.00		y =	-7.38E-02	+	1.58E-01	x		0.00 - 50.00	
19-Norethindron	y =	-1.09E-01	+	1.97E-01	x		0.00 - 50.00		y =	-5.91E-02	+	1.79E-01	x		0.00 - 40.00	
Methylboldenone	y =	-4.52E-02	+	1.09E-01	x		0.00 - 40.00		y =	-4.80E-02	+	1.19E-01	x		0.00 - 40.00	
11-ketotestosterone	y =	9.67E-03	+	1.10E-01	x		0.00 - 50.00		y =	3.97E-02	+	1.09E-01	x		0.00 - 40.00	
Formestane	y =	3.35E-04	+	5.91E-02	x		0.00 - 50.00		y =	6.24E-03	+	8.68E-02	x		0.00 - 40.00	

Analyte	Seawater						Fresh water					
	Equation					Dynamic range ($ng\ L^{-1}$)	Equation					Dynamic range ($ng\ L^{-1}$)
Norethandrolone	y =	2.90E-01	+	4.97E-01	x	0.00 - 40.00	y =	2.06E-01	+	5.80E-01	x	0.00 - 40.00
Methyltestosterone	y =	-1.26E-02	+	1.72E-01	x	0.00 - 50.00	y =	-8.99E-03	+	1.51E-01	x	0.00 - 40.00
Trenbolone acetate	y =	4.25E-01	+	2.70E-01	x	0.00 - 50.00	y =	-7.87E-02	+	1.68E-01	x	0.00 - 50.00
Ethynyl testosterone	y =	-3.20E-01	+	5.55E-01	x	0.00 - 50.00	y =	-1.84E-01	+	3.94E-01	x	0.00 - 50.00
Stanozolol	y =	-3.98E-02	+	9.98E-01	x	0.00 - 50.00	y =	-1.26E-01	+	1.34E-01	x	0.00 - 40.00
Testosterone acetate	y =	-2.38E-01	+	2.51E-01	x	0.00 - 40.00	y =	-9.19E-03	+	2.36E-01	x	0.00 - 40.00
Fluoxymesterone	y =	-1.20E-01	+	7.83E-02	x	0.00 - 50.00	y =	-4.61E-02	+	7.76E-02	x	0.00 - 40.00
Testosterone propionate	y =	-1.43E-01	+	2.60E-01	x	0.00 - 50.00	y =	-6.13E-02	+	2.08E-01	x	0.00 - 40.00
Chlorotestosteron acetate	y =	-4.64E-02	+	1.43E-01	x	0.00 - 50.00	y =	-7.00E-02	+	1.28E-01	x	0.00 - 50.00
Testosterone benzoate	y =	-1.46E-01	+	1.43E-01	x	0.00 - 50.00	y =	-1.15E-01	+	9.83E-02	x	0.00 - 50.00
Testosterone phenylpropionate	y =	-4.19E-02	+	1.16E-01	x	0.00 - 50.00	y =	-2.73E-01	+	1.31E-01	x	0.00 - 50.00
19-nortestosterone-17-decanoate	y =	6.19E-02	+	4.92E-02	x	0.00 - 40.00	y =	-7.24E-02	+	3.69E-02	x	0.00 - 50.00
Oestrogens												
17α-estradiol	y =	-3.14E-01	+	1.59E-01	x	0.00 - 50.00	y =	-1.57E-01	+	1.49E-01	x	0.00 - 50.00
17β-estradiol	y =	1.33E-01	+	1.19E-02	x	0.00 - 50.00	y =	-4.81E-02	+	1.58E-02	x	0.00 - 50.00
Estradiol-17-acetate	y =	-3.64E-02	+	7.57E-02	x	0.00 - 40.00	y =	-7.39E-02	+	6.80E-02	x	0.00 - 40.00
Dienoestrol	y =	-1.23E-02	+	2.24E-01	x	0.00 - 50.00	y =	4.39E-02	+	2.34E-01	x	0.00 - 40.00
Equilin	y =	2.57E-01	+	9.68E-02	x	0.00 - 50.00	y =	-5.91E-02	+	1.09E-01	x	0.00 - 40.00
Diethylstilbestrol	y =	3.03E-01	+	9.21E-02	x	0.00 - 50.00	y =	-6.18E-02	+	1.08E-01	x	0.00 - 40.00
Estrone	y =	-1.05E-02	+	2.29E-01	x	0.00 - 50.00	y =	-2.17E-01	+	2.68E-01	x	0.00 - 50.00
17α-ethinylestradiol	y =	-1.63E-01	+	1.24E-01	x	0.00 - 50.00	y =	-2.27E-01	+	1.22E-01	x	0.00 - 50.00
α-zearalenol	y =	-5.68E-02	+	1.05E-01	x	0.00 - 50.00	y =	1.49E-01	+	1.29E-01	x	0.00 - 50.00

Analyte	Seawater						Fresh water					
	Equation					Dynamic range (ng L^{-1})	Equation					Dynamic range (ng L^{-1})
β-zearalenol	y =	-4.72E-01	+	9.21E-02	x	0.00 - 50.00	y =	1.40E+00	+	4.26E-02	x	0.00 - 50.00
α-zeranol	y =	-1.89E-02	+	1.33E-01	x	0.00 - 50.00	y =	6.75E-01	+	1.75E-01	x	0.00 - 50.00
β-zeranol	y =	-2.11E-04	+	9.80E-02	x	0.00 - 50.00	y =	3.97E-02	+	1.09E-01	x	0.00 - 50.00
Gestodene	y =	2.27E-02	+	5.32E-01	x	0.00 - 40.00	y =	-5.08E-01	+	7.10E-01	x	0.00 - 50.00
Estradiol-benzoate	y =	-6.25E-02	+	2.89E-01	x	0.00 - 50.00	y =	-1.70E-01	+	2.32E-01	x	0.00 - 40.00
Progestins												
5α-Pregnan-3α,20β-diol	y =	-1.20E-01	+	3.92E-02	x	0.00 - 50.00	y =	-2.26E-02	+	3.01E-02	x	0.00 - 50.00
Norgestrel	y =	-1.82E-01	+	5.45E-01	x	0.00 - 50.00	y =	-2.66E-01	+	6.26E-01	x	0.00 - 40.00
Dihydroprogesterone	y =	-1.59E-01	+	2.76E-01	x	0.00 - 40.00	y =	-1.52E-01	+	3.27E-01	x	0.00 - 40.00
Progesterone	y =	-4.90E-01	+	7.11E-01	x	0.00 - 50.00	y =	-4.84E-01	+	5.18E-01	x	0.00 - 40.00
Methylprogesterone	y =	-8.28E-01	+	8.22E-01	x	0.00 - 50.00	y =	-4.38E-02	+	4.50E-01	x	0.00 - 40.00
17α-hydroxyprogesterone	y =	-1.09E-01	+	2.05E-01	x	0.00 - 40.00	y =	-1.79E-01	+	2.51E-01	x	0.00 - 40.00
Megestrol	y =	-2.39E-01	+	1.96E-01	x	0.00 - 40.00	y =	-6.85E-02	+	2.07E-01	x	0.00 - 40.00
Medroxyprogesterone	y =	-7.93E-02	+	2.13E-01	x	0.00 - 50.00	y =	-1.55E-01	+	2.50E-01	x	0.00 - 50.00
17α-acetoxyprogesterone	y =	-3.27E-01	+	1.93E-01	x	0.00 - 50.00	y =	-4.33E-02	+	1.68E-01	x	0.00 - 40.00
Megestrol acetate	y =	-1.04E-01	+	1.75E-01	x	0.00 - 50.00	y =	-7.87E-02	+	1.68E-01	x	0.00 - 50.00
Medroxyprogesterone acetate	y =	-2.49E-02	+	8.58E-02	x	0.00 - 50.00	y =	-5.75E-02	+	7.95E-02	x	0.00 - 40.00
Flugestone acetate	y =	-7.73E-02	+	7.47E-02	x	0.00 - 50.00	y =	-1.59E-01	+	1.02E-01	x	0.00 - 50.00
Caproxyprogesterone	y =	-3.02E-01	+	2.99E-01	x	0.00 - 40.00	y =	-1.43E-01	+	1.66E-01	x	0.00 - 40.00
Corticosteroids												
Prednisone	y =	-1.19E-01	+	1.68E-02	x	0.00 - 50.00	y =	6.51E-02	+	1.28E-02	x	0.00 - 50.00
Corticosterone	y =	-7.88E-02	+	2.83E-02	x	0.00 - 40.00	y =	-9.33E-04	+	3.37E-02	x	0.00 - 40.00

Analyte	Seawater						Fresh water					
	Equation					Dynamic range (ng L^{-1})	Equation					Dynamic range (ng L^{-1})
Cortisone	y =	-2.11E-03	+	9.16E-03	x	0.00 - 40.00	y =	3.86E-02	+	1.60E-02	x	0.00 - 40.00
Prednisolone	y =	-6.76E-02	+	1.38E-02	x	0.00 - 50.00	y =	-5.73E-04	+	1.19E-02	x	0.00 - 50.00
Cortisol	y =	-3.45E-03	+	1.02E-02	x	0.00 - 50.00	y =	5.77E-03	+	1.47E-02	x	0.00 - 50.00
Tetrahydrocortisone	y =	-8.35E-02	+	4.30E-02	x	0.00 - 50.00	y =	8.43E-03	+	4.04E-02	x	0.00 - 40.00
Corticosterone acetate	y =	-8.32E-03	+	2.89E-02	x	0.00 - 50.00	y =	-3.25E-02	+	3.97E-02	x	0.00 - 50.00
Dexamethasone	y =	-1.21E-01	+	1.37E-02	x	0.00 - 50.00	y =	-2.21E-03	+	9.50E-03	x	0.00 - 40.00
Prednisolone acetate	y =	-4.36E-02	+	1.38E-01	x	0.00 - 50.00	y =	-1.76E-02	+	2.17E-02	x	0.00 - 50.00
Cortisone acetate	y =	8.09E-02	+	5.88E-04	x	0.00 - 50.00	y =	5.09E-03	+	2.33E-02	x	0.00 - 50.00
Hydrocortisone 21-acetate	y =	-1.52E-02	+	2.26E-02	x	0.00 - 50.00	y =	-1.20E-01	+	2.54E-02	x	0.00 - 50.00

Table A5. Summary of results for the cross-validation on fresh water.

Analyte	Recovery (%)	Limits		Precision	Linearity
		MDL ($ng\ L^{-1}$)	MQL ($ng\ L^{-1}$)	Repeatability RSD (%)	R^2
Androgens					
Methandriol	99.3 + 5.8	0.13	0.50	5.5	0.9972
17α-trenbolone	95.1 + 4.7	0.25	0.50	5.0	0.9984
17β-trenbolone	101.8 + 2.4	0.25	0.50	2.4	0.9974
11β-hydroxyandrosterone	100.7 + 2.4	0.25	0.50	2.4	0.9983
Testosterone cypionate	100.4 + 4.1	0.25	0.50	4.1	0.9922
17β-dihydroandrosterone	98.2 + 5.3	0.13	0.50	5.8	0.9974
Androsterone	99.0 + 2.0	0.25	1.00	2.0	0.9919
19-nortestosterone	98.2 + 4.3	0.13	1.00	5.0	0.9927
1,4-Androstadienedione	102.1 + 6.5	0.13	0.50	5.4	0.9940
11-ketoetiocholanolone	100.2 + 6.9	0.13	0.50	6.8	0.9974
Androstenedione	96.5 + 6.1	0.25	0.50	5.8	0.9937
Mestanolone	97.7 + 8.5	0.50	1.00	8.7	0.9917
17α-testosterone	100.4 + 3.1	0.13	0.25	3.1	0.9946
17β-testosterone	98.1 + 1.9	0.06	0.25	2.0	0.9970
5α-dihydrotestosterone	99.2 + 5.8	0.13	0.25	4.8	0.9987
19-Norethindron	99.5 + 6.1	0.50	0.75	6.3	0.9968
Methylboldenone	97.3 + 8.2	0.50	1.00	7.8	0.9952
11-ketotestosterone	103.7 + 4.5	0.13	0.50	4.9	0.9992
Formestane	99.3 + 9.0	0.25	1.00	9.2	0.9903
Norethandrolone	97.3 + 2.2	0.06	0.13	2.3	0.9969
Methyltestosterone	100.2 + 2.6	0.13	0.25	2.6	0.9953
Trenbolone acetate	100.0 + 1.7	0.25	0.75	1.7	0.9967
Ethynyl testosterone	102.8 + 5.8	0.13	0.50	4.9	0.9937
Stanozolol	99.2 + 4.4	1.00	2.50	4.4	0.9930
Testosterone acetate	98.8 + 6.7	0.13	0.75	6.9	0.9947
Fluoxymesterone	102.5 + 3.7	0.50	1.00	3.6	0.9942
Testosterone propionate	99.0 + 4.0	0.13	0.25	4.1	0.9946
Chlorotestosteron acetate	100.2 + 8.4	0.25	0.75	8.4	0.9963
Testosterone benzoate	99.4 + 2.5	0.50	0.75	2.5	0.9900
Testosterone phenylpropionate	96.0 + 6.8	0.25	0.75	7.1	0.9902
19-nortestosterone-17-decanoate	99.1 + 2.4	2.50	5.00	2.4	0.9956
Oestrogens					
17α-estradiol	99.1 + 3.7	0.75	0.75	3.8	0.9964

Analyte	Recovery (%)	Limits		Precision	Linearity
		MDL ($ng\ L^{-1}$)	MQL ($ng\ L^{-1}$)	Repeatability RSD (%)	R^2
17β-estradiol	98.0 + 7.2	0.13	0.25	5.2	0.9345
Estradiol-17-acetate	96.4 + 5.5	0.50	1.00	5.7	0.9920
Dienoestrol	99.3 + 1.6	0.25	1.00	1.6	0.9960
Equilin	100.0 + 5.4	0.13	0.25	4.9	0.9945
Diethylstilbestrol	98.3 + 2.8	0.25	5.00	2.8	0.9947
Estrone	101.9 + 4.6	0.13	0.50	5.2	0.9965
17α-ethinylestradiol	103.8 + 7.2	2.50	5.00	7.6	0.9983
α-zearalenol	101.9 + 2.9	0.25	5.00	2.8	0.9970
β-zearalenol	102.4 + 2.0	0.13	0.75	2.1	0.9921
α-zeranol	102.7 + 1.8	0.13	0.75	1.8	0.9928
β-zeranol	106.9 + 8.7	5.00	5.00	11.2	0.9903
Gestodene	97.0 + 7.0	0.50	2.50	7.2	0.9967
Estradiol-benzoate	95.0 + 9.2	1.00	1.00	9.7	0.9921
Progestins					
5α-Pregnan-3α,20β-diol	96.6 + 8.0	2.50	5.00	8.3	0.9914
Norgestrel	99.2 + 2.2	0.06	0.25	2.2	0.9954
Dihydroprogesterone	101.6 + 3.2	0.13	0.50	3.1	0.9970
Progesterone	97.3 + 2.6	0.06	0.50	2.7	0.9978
Methylprogesterone	103.6 + 4.9	0.06	0.25	4.7	0.9977
17α-hydroxyprogesterone	98.5 + 2.5	0.13	0.50	2.5	0.9940
Megestrol	97.6 + 6.7	0.25	0.50	6.8	0.9923
Medroxyprogesterone	99.8 + 2.4	0.25	0.50	2.4	0.9941
17α-acetoxyprogesterone	93.0 + 5.2	0.13	1.00	5.3	0.9926
Megestrol acetate	96.8 + 4.8	0.25	0.75	4.9	0.9978
Medroxyprogesterone acetate	100.6 + 3.2	0.50	0.75	4.8	0.9987
Flugestone acetate	103.2 + 8.1	0.75	1.00	7.6	0.9901
Caproxyprogesterone	98.9 + 3.8	0.25	0.75	3.3	0.9928
Corticosteroids					
Prednisone	103.4 + 3.9	0.13	0.25	3.8	0.9935
Corticosterone	101.9 + 4.3	0.25	0.75	4.2	0.9927
Cortisone	103.8 + 4.5	0.25	5.00	4.3	0.9969
Prednisolone	99.7 + 2.6	0.25	0.50	2.2	0.9904
Cortisol	98.3 + 3.1	0.25	2.50	2.6	0.9979
Tetrahydrocortisone	107.2 + 6.8	0.25	0.50	5.3	0.9977
Corticosterone acetate	100.2 + 3.4	2.50	2.50	3.8	0.9969
Dexamethasone	97.9 + 8.1	2.50	5.00	8.2	0.9996

Analyte	Recovery (%)	Limits		Precision	Linearity
		MDL ($ng\ L^{-1}$)	MQL ($ng\ L^{-1}$)	Repeatability RSD (%)	R^2
Prednisolone acetate	98.0 + 8.0	2.50	2.50	8.3	0.9907
Cortisone acetate	101.5 + 1.7	0.06	0.50	1.6	0.9906
Hydrocortisone 21-acetate	98.0 + 6.7	5.00	5.00	5.8	0.9947

APPENDIX B - TARGETED QUANTIFICATION AND UNTARGETED SCREENING OF ALKYLPHENOLS, BISPHENOL A AND PHTHALATES IN AQUATIC MATRICES USING ULTRA-HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY COUPLED TO HYBRID Q-ORBITRAP MASS SPECTROMETRY

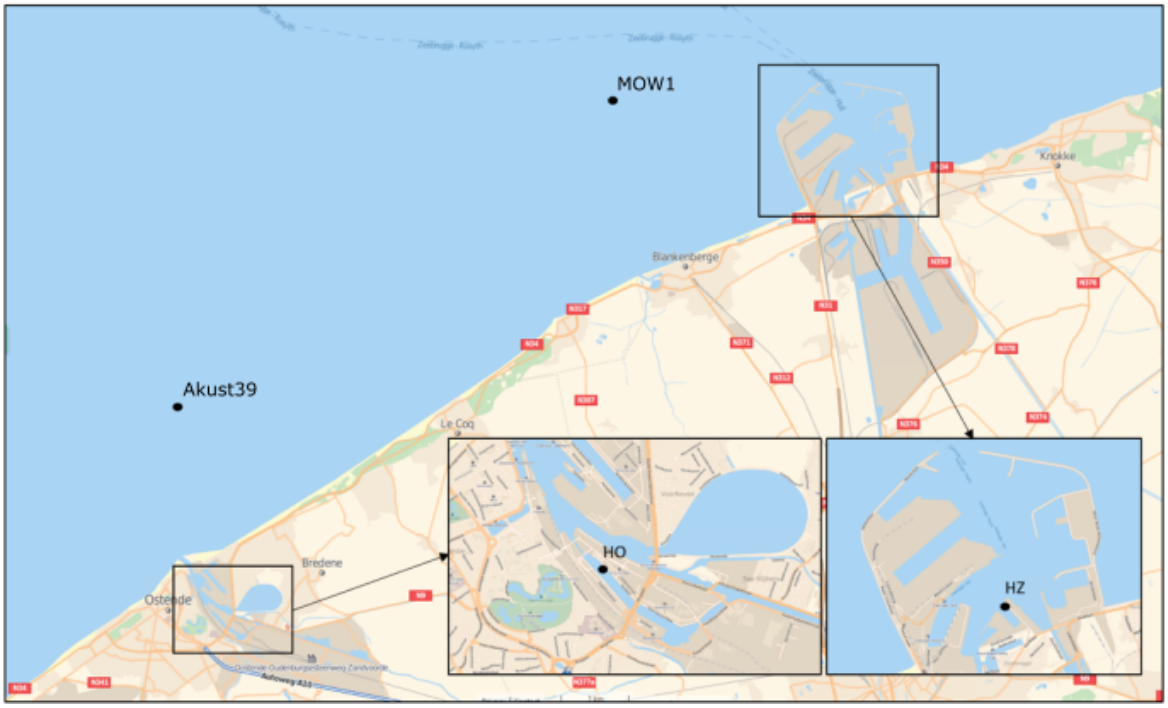


Figure B1. The 4 sampling locations that were monitored in the BPNS during the winter of 2016 and spring 2017, i.e. 51°21'37.78"N; 3° 6'49.01"O (MOW1), 51°20'25.68"N; 3°12'12.11"O (HZ), 51°14'48.59"N; 2°55'39.61"O (Akust39) and 51°13'34.68"N; 2°56'8.00"O (HO).

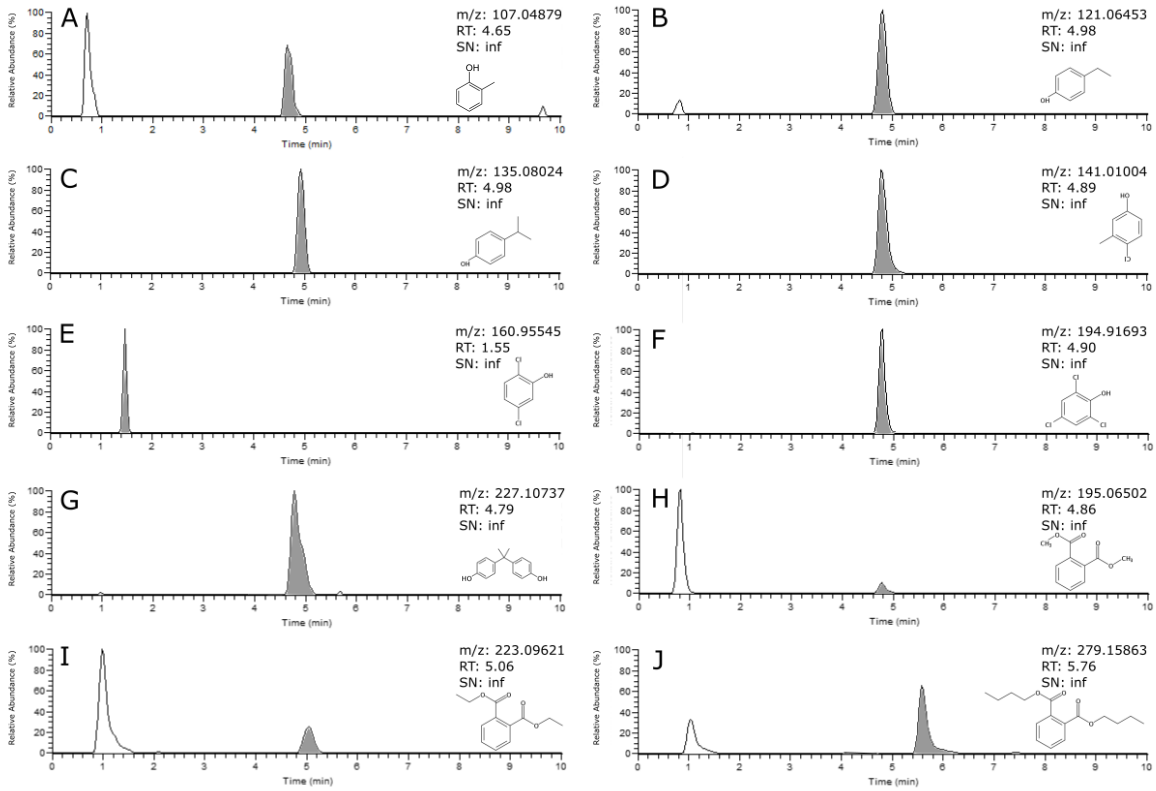


Figure B2. UHPLC-HR-Q-Orbitrap-MS chromatograms of the instrumental method at 10 ng on column for each target compound (PART I), i.e. 2-methyl phenol (a), 4-ethyl phenol (b), 4-isopropyl phenol (c), 4-chloro-3-methylphenol (d), 2,5-dichlorophenol (e), 3,6,6-trichlorophenol (f), Bisphenol A (g), dimethyl phthalate (h), diethyl phthalate (i) and dibutyl phthalate (j).

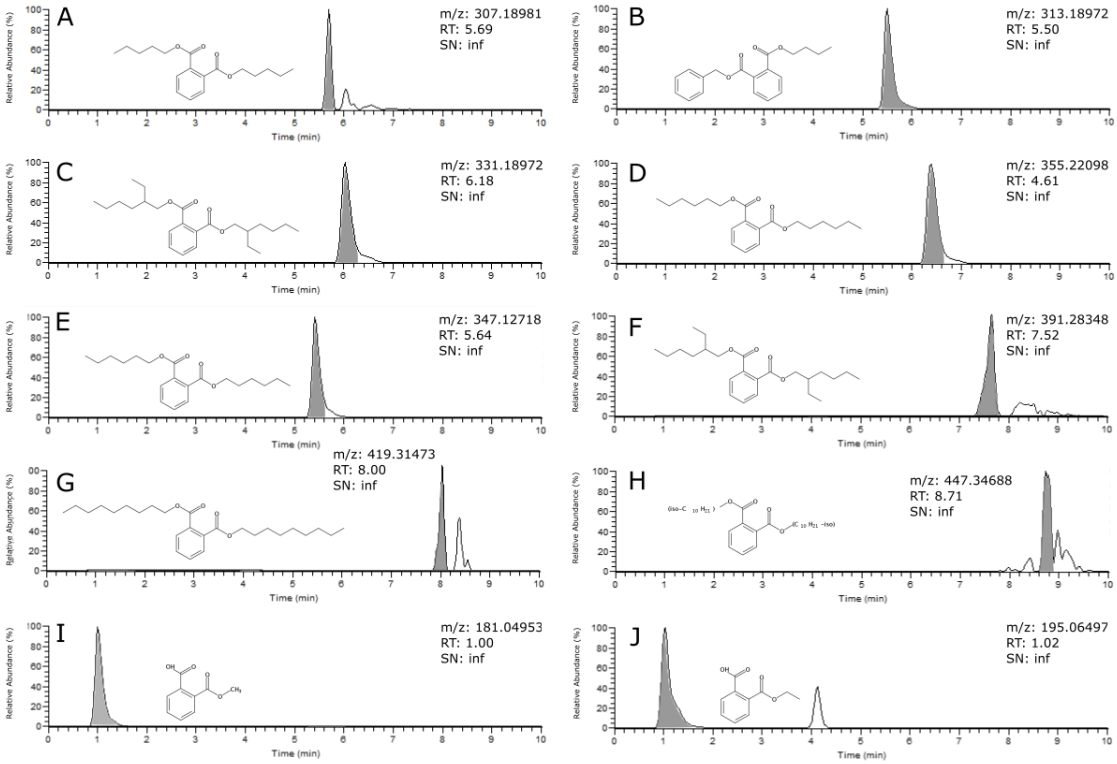


Figure B3. UHPLC-HR-Q-Orbitrap-MS chromatograms of the instrumental method at 10 ng on column for each target compound (PART II), i.e. diamyl phthalate (a), benzyl butyl phthalate (b), dicyclohexyl phthalate (c), dihexyl phthalate (d), dibenzyl phthalate (e), diethylhexyl phthalate (f), dinonyl phthalate (g), diisodecyl phthalate (h), monomethyl phthalate (i) and monoethyl phthalate (j).

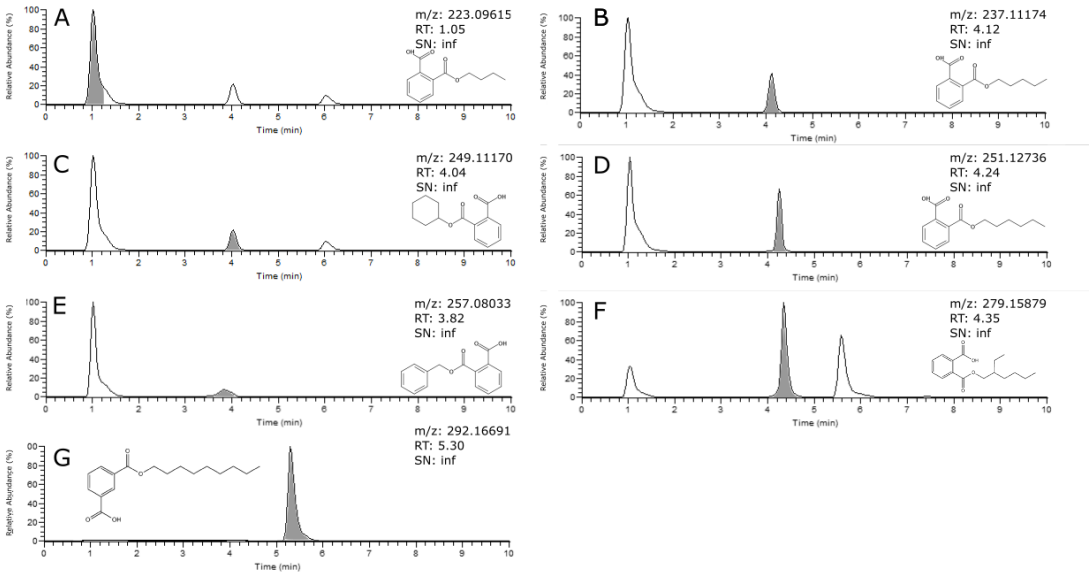


Figure B4. UHPLC-HR-Q-Orbitrap-MS chromatograms of the instrumental method at 10 ng on column for each target compound (PART III), i.e. monobutyl phthalate (a), mono-n-pentyl phthalate (b), monocyclohexyl phthalate (c), monohexyl phthalate (d), monobenzyl phthalate (e), monoethylhexyl phthalate (f) and mono-isonyl phthalate (g).

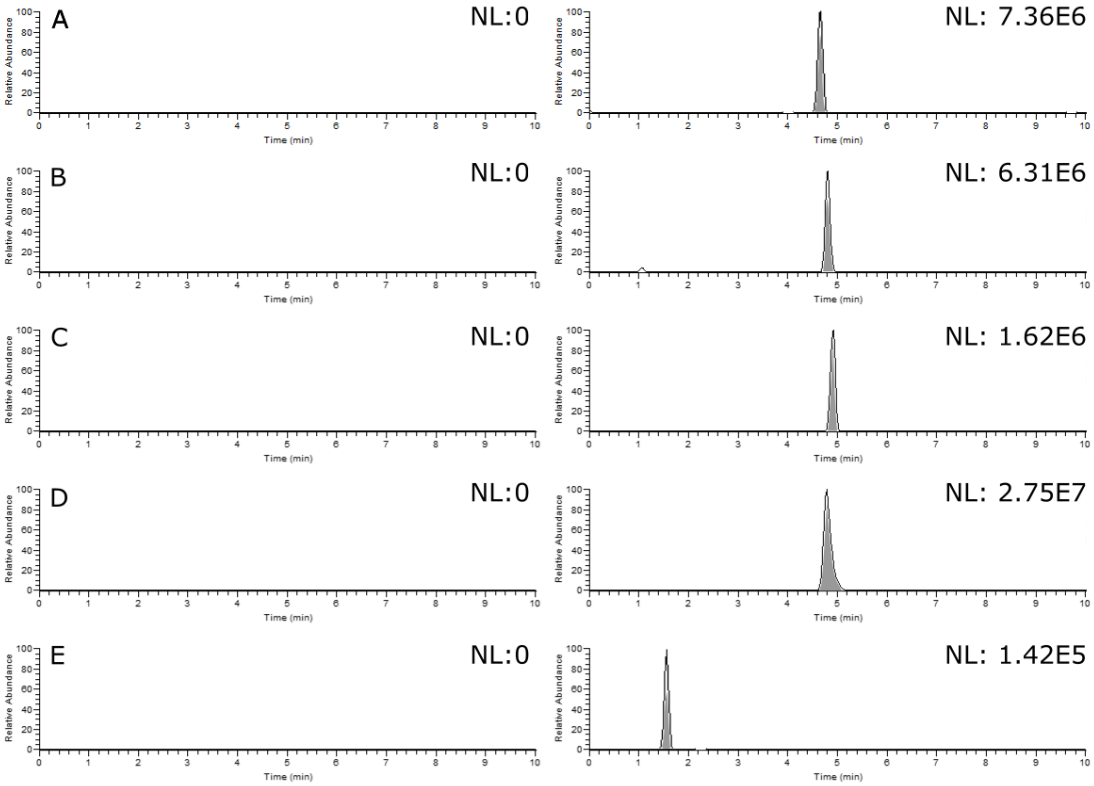


Figure B5. UHPLC-HR-Q-Orbitrap-MS chromatograms of the blank (left) and spiked reference seawater (right) after the optimised extraction procedure at 1.5 times the MQL-level (PART I), i.e. 2-methyl phenol (a), 4-ethyl phenol (b), 4-isopropyl phenol (c), 4-chloro-3-methylphenol (d) and 2,5-dichlorophenol (e). NL represents the noise level.

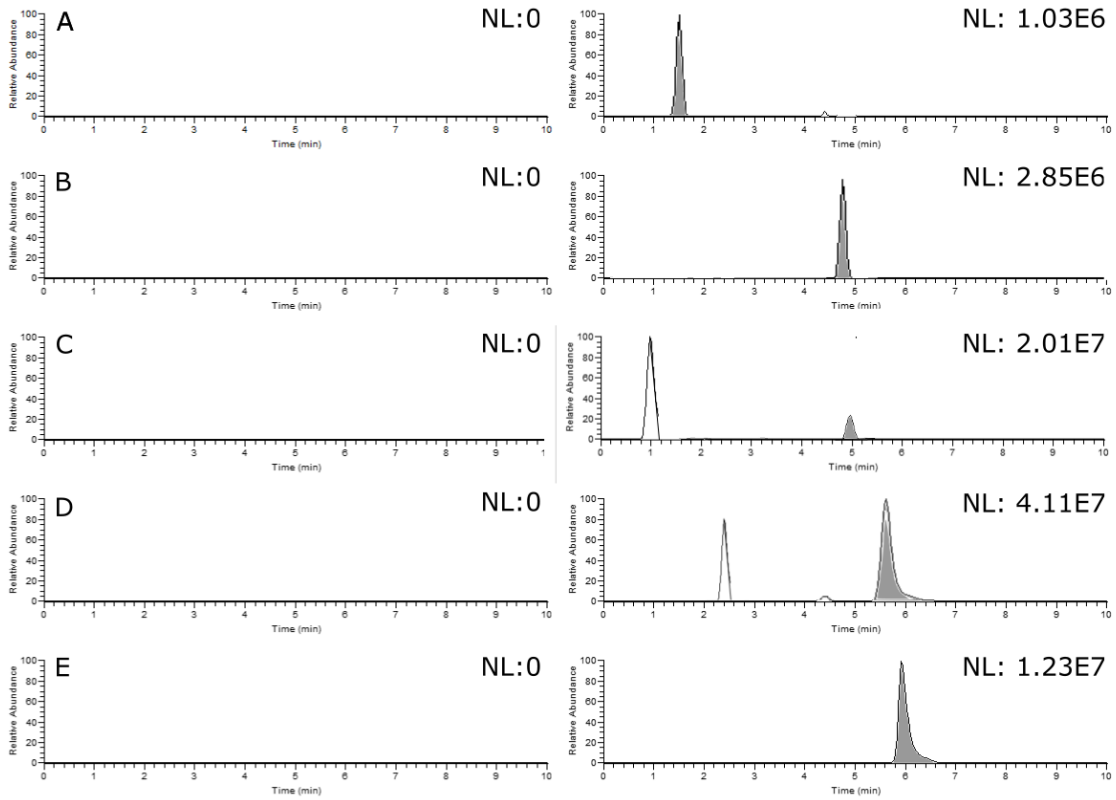


Figure B6. UHPLC-HR-Q-Orbitrap-MS chromatograms of the blank (left) and spiked reference seawater (right) after the optimised extraction procedure at 1.5 times the MQL-level (PART II), i.e. 3,4,6-trichlorophenol (a), Bisphenol A (b), dimethyl phthalate (c), diethyl phthalate (d) and dibutyl phthalate (e). NL represents the noise level.

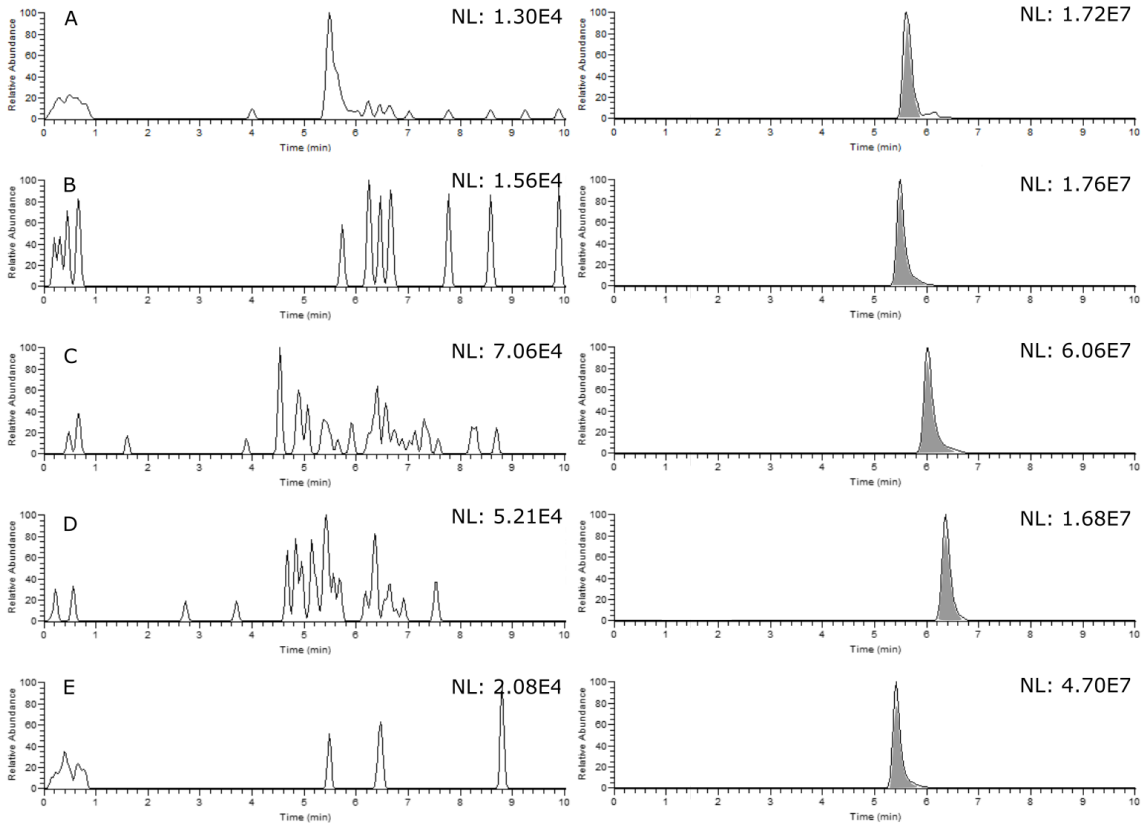


Figure B7. UHPLC-HR-Q-Orbitrap-MS chromatograms of the blank (left) and spiked reference seawater (right) after the optimised extraction procedure at 1.5 times the MQL-level (PART III), i.e. diamyl phthalate (a), benzyl butyl phthalate (b), dicyclohexyl phthalate (c), dihexyl phthalate (d) and dibenzyl phthalate (e). NL represents the noise level.

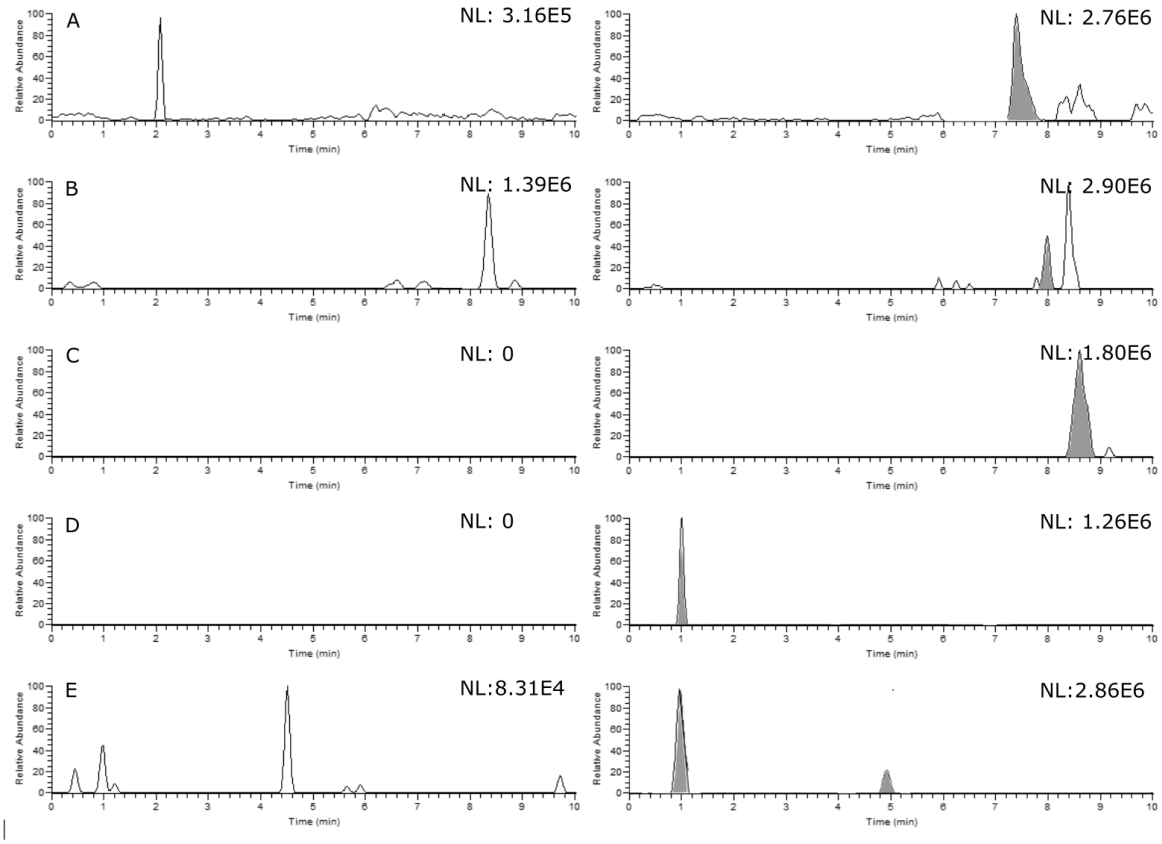


Figure B8. UHPLC-HR-Q-Orbitrap-MS chromatograms of the blank (left) and spiked reference seawater (right) after the optimised extraction procedure at 1.5 times the MQL-level (PART IV), i.e. diethylhexyl phthalate (a), dinonyl phthalate (b), diisodecyl phthalate (c), monomethyl phthalate (d) and mo-noethyl phthalate (e). NL represents the noise level.

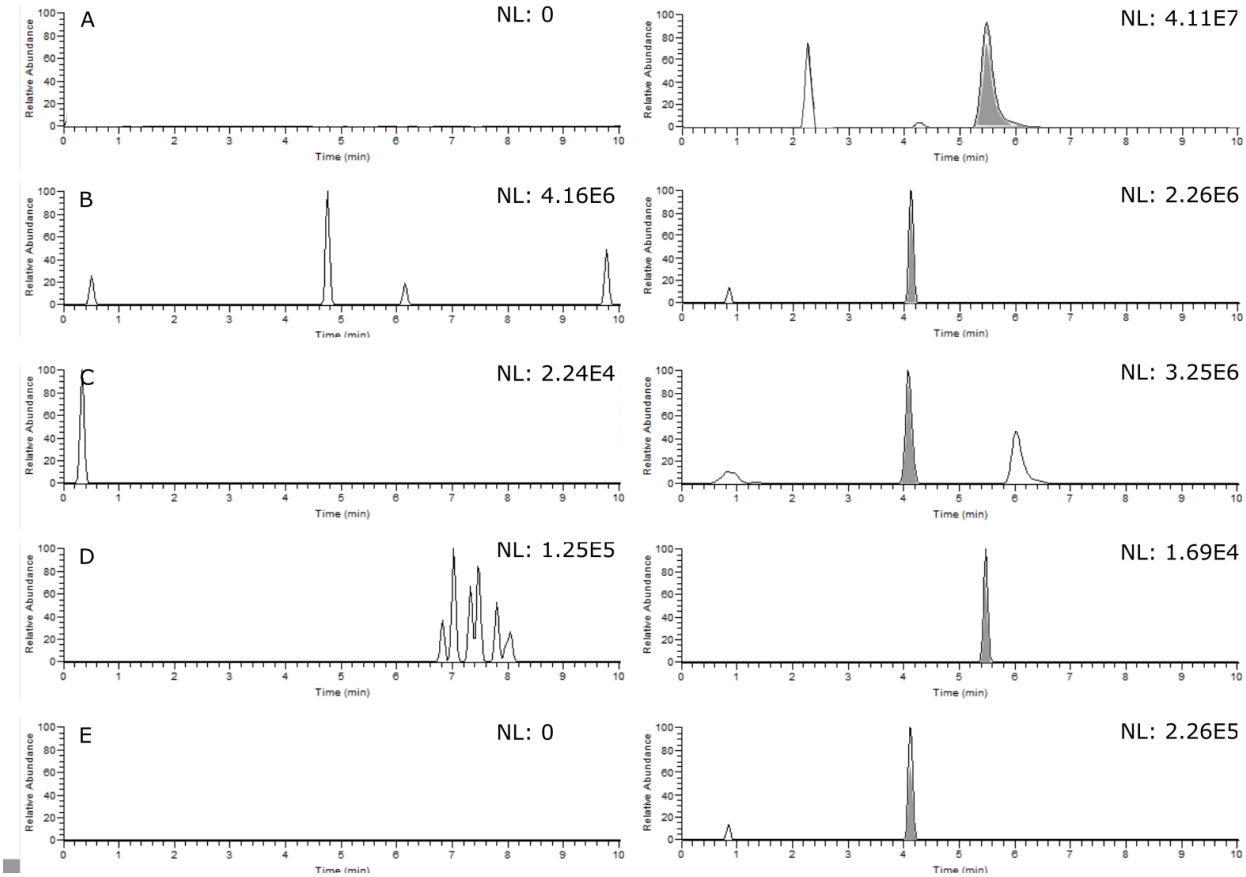


Figure B9. UHPLC-HR-Q-Orbitrap-MS chromatograms of the blank (left) and spiked reference seawater (right) after the optimised extraction procedure at 1.5 times the MQL-level (PART V), i.e. monobutyl phthalate (a), mono-n-pentyl phthalate (b), monocyclohexyl phthalate (c), monohexyl phthalate (d) and monobenzyl phthalate (e). NL represents the noise level.

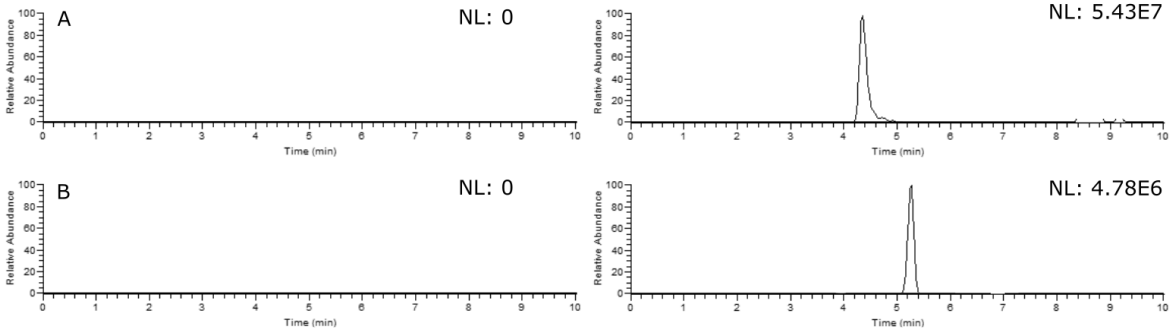


Figure B10. UHPLC-HR-Q-Orbitrap-MS chromatograms of the blank (left) and spiked reference seawater (right) after the optimised extraction procedure at 1.5 times the MQL-level (PART VI), i.e. monoethylhexyl phthalate (f) and mono-isonyl phthalate (g). NL represents the noise level.

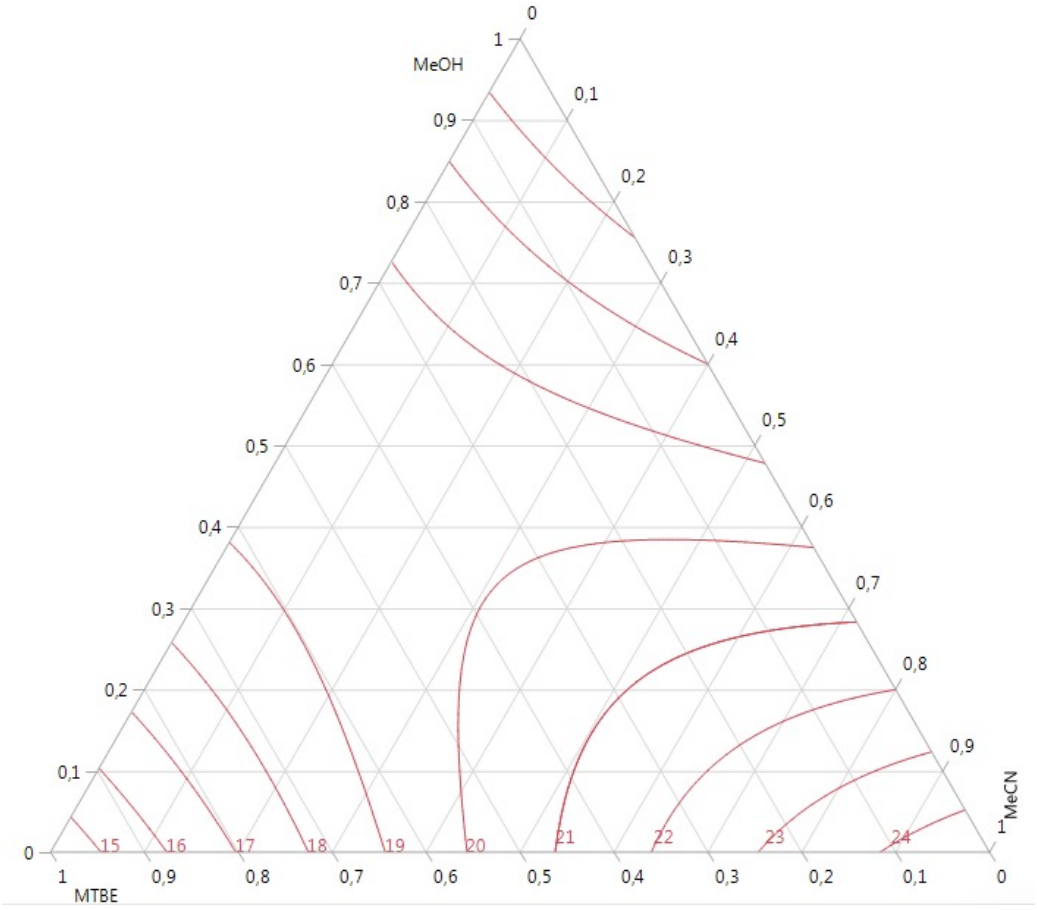


Figure B11. Ternary plot showing the optimal SPE eluent composition according to the simplex lattice design.

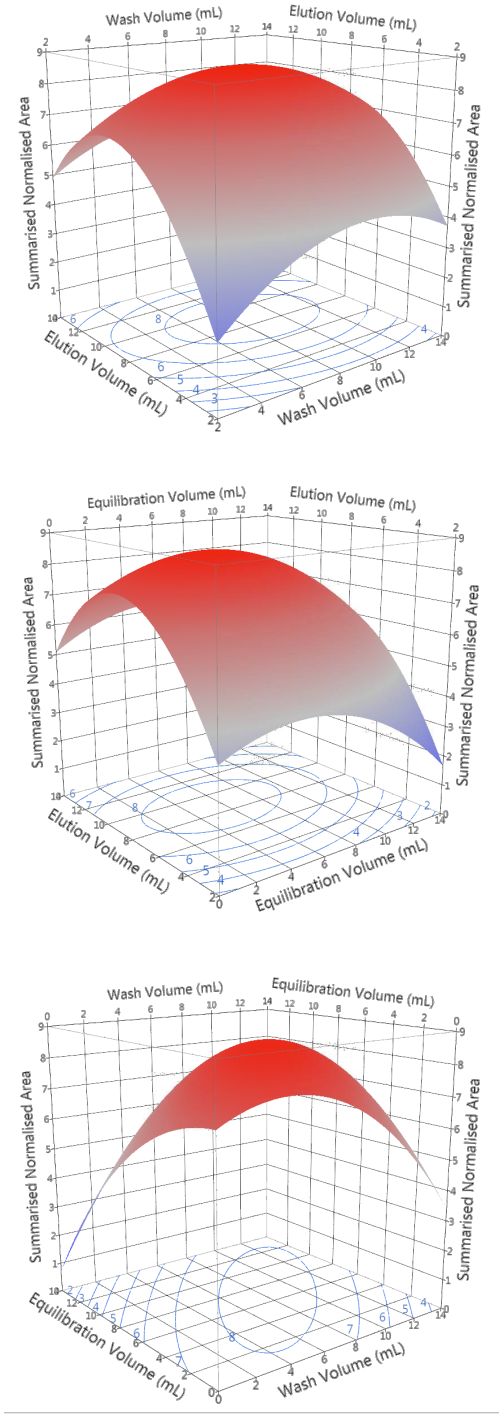


Figure B12. Response surface plots showing the highest response during SPE optimization according to the box-behnken design.

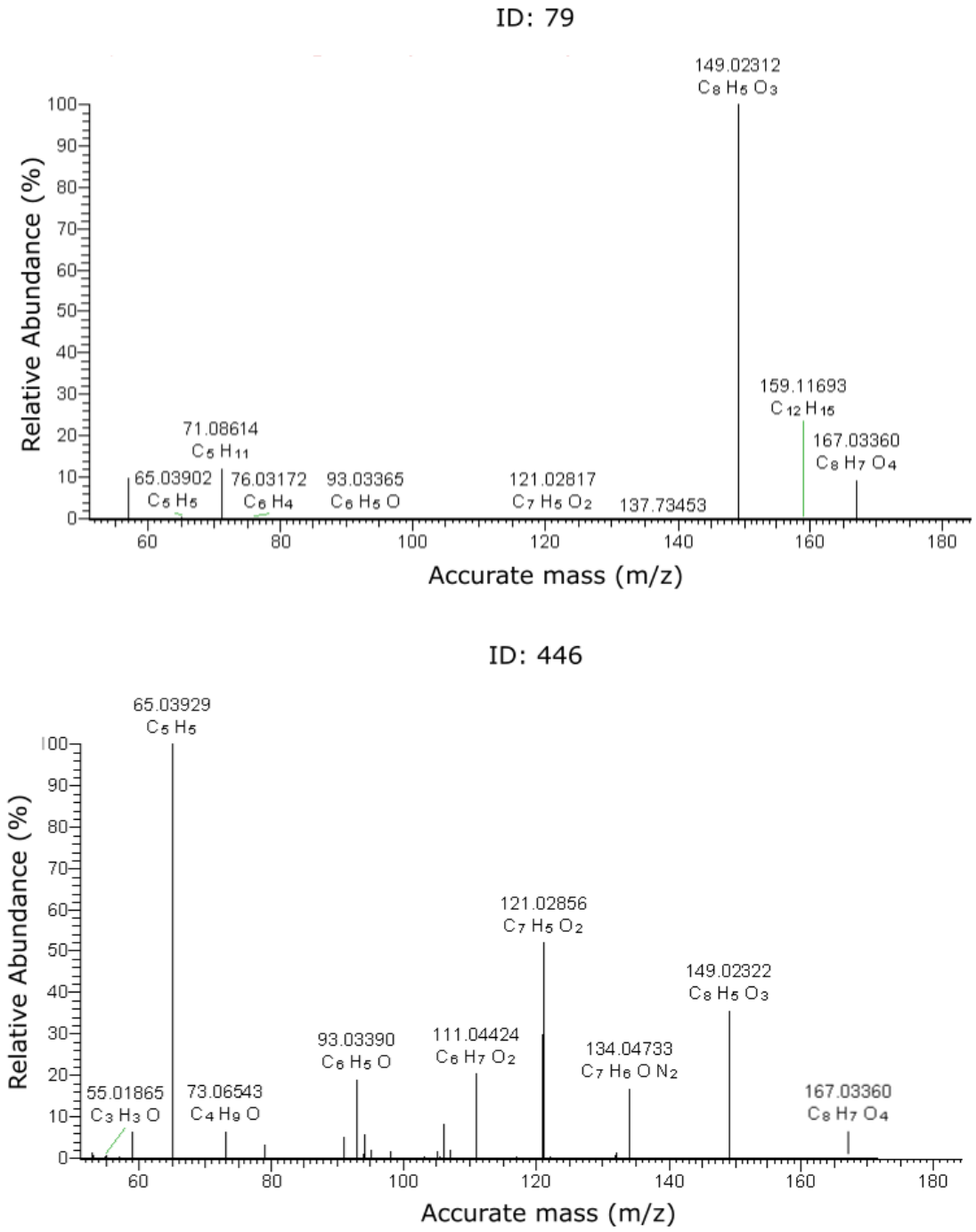


Figure B13. Selected MS/MS spectra obtained by performing the fragmentation of identifier (ID) 79 and 446, which shows the specific characteristic fragments for phthalates.

Table B1. The target AP and PAE compounds including CAS number, molecular mass (g mol^{-1}), pKa, log P, water solubility (mg L^{-1}), vapor pressure (Torr) and bioconcentration factor (L kg^{-1}) at a temperature of 25°C and pH 7 (data obtained from csifinder.cas.org).

Compound	CAS number	Molecular mass	pKa	Log P	Water solubility	Vapor Pressure	Bioconcentration
Phenols							
2-methyl phenol	106-44-5	108.14	10.21 ± 0.13	2.066 ± 0.192	2.20E+04	2.11E-01	2.19E+01
4-ethylphenol	123-07-9	122.16	10.26 ± 0.13	2.576 ± 0.192	6.00E+03	8.28E-02	5.34E+01
4-isopropyl phenol	99-89-8	136.19	10.19 ± 0.13	2.986 ± 0.200	2.00E+03	1.47E-01	1.09E+02
4-chloro-3-methylphenol	59-50-7	142.58	9.63 ± 0.18	2.894 ± 0.223	9.80E+02	3.35E-02	9.29E+01
2,5-dichlorophenol	583-78-8	163.00	7.53 ± 0.1	3.025 ± 0.237	4.70E+02	1.06E-01	9.06E+01
3,4,6-trichlorophenol	88-06-2	197.45	6.59 ± 0.23	3.769 ± 0.327	9.30E+01	1.77E-02	1.21E+02
Bisphenol A	65-85-0	228.29	4.2 ± 0.1	1.559 ± 0.206	5.60E+03	1.22E-02	1.00E+00
Di-phthalates							
Dimethyl phthalate	131-11-3	194.18	-	1.695 ± 0.253	2.50E+03	3.31E-03	1.14E+01
Diethyl phthalate	84-66-2	222.24	-	2.714 ± 0.253	5.30E+02	1.67E-03	6.80E+01
Dibutyl phthalate	84-74-2	278.34	-	4.752 ± 0.254	2.50E+01	1.08E-04	2.41E+03
Diamyl phthalate	131-18-0	306.40	-	5.771 ± 0.254	5.80E+00	2.80E-05	1.43E+04
Benzyl butyl phthalate	85-68-7	312.36	-	4.910 ± 0.266	7.50E+00	7.09E-07	3.17E+03
Dicyclohexyl phthalate	84-61-7	330.42	-	5.639 ± 0.257	3.20E+00	1.87E-07	1.14E+04
Dihexyl phthalate	84-75-3	334.45	-	6.790 ± 0.254	1.40E+00	6.93E-06	8.51E+04
Dibenzyl phthalate	523-31-9	346.38	-	5.067 ± 0.353	2.10E+00	4.90E-09	4.18E+03
Diethylheyxl phthalate	117-81-7	390.56	-	8.516 ± 0.261	1.10E-01	3.95E-06	1.00E+06
Dinonyl phthalate	84-76-4	418.61	-	9.847 ± 0.254	2.50E-02	8.72E-08	1.00E+06
Diisodecyl phthalate	26761-40-0	446.66	-	-	-	-	-
Mono-phthalates							
Monomethyl phthalate	4376-18-5	180.16	3.32 ± 0.1	1.130 ± 0.230	5.60E+03	7.39E-05	1.00E+00
Monoethyl phthalate	2306-33-4	194.18	3.32 ± 0.1	1.639 ± 1.639	2.70E+03	3.53E-05	1.00E+00
Monotbutyl phthalate	131-70-4	222.24	3.38 ± 0.36	2.658 ± 2.658	6.70E+02	6.40E-06	1.00E+00
Mono-n-pentyl phthalate	24539-56-8	236.26	3.38 ± 0.36	3.168 ± 0.231	3.50E+02	2.53E-06	1.00E+00
Monocyclohexyl pht.	7517-36-4	248.27	3.29 ± 0.36	3.102 ± 0.233	3.00E+02	1.82E-07	1.00E+00
Monohexyl phthalate	24539-57-9	250.29	3.39 ± 0.36	3.677 ± 0.231	2.00E+02	9.66E-07	1.00E+00
Monobenzyl phthalate	2528-16-7	256.25	3.37 ± 0.36	2.816 ± 0.246	2.50E+02	1.48E-08	1.00E+00
Monoethylhexyl phthalate	4376-20-9	278.34	3.37 ± 0.36	4.451 ± 0.235	7.80E+01	2.03E-07	1.57E+00
Mono-isonyl phthalate	68515-53-7	-	-	-	-	-	-

Table B2. Screening parameters with their specific ranges for the optimization of the extraction of APs and PAEs from seawater. Parameters are listed in the order that they occur during execution of the extraction procedure.

Parameters	Type of variable	Unit	Ranges		
			Lower level (-1)	Central level (0)	Upper level (+1)
Pretreatment	Categorical	/	Filter	/	No filter
pH adjustement sample	Continue	/	3.0	5.5	8.0
SPE-cartridge	Categorical	/	Oasis™ HLB	/	Strata X™
Conditioning solvent solvent	Categorical	/	5% CH_3CN	/	5% CH_3OH
Volume conditioning solvent	Continue	mL	3	4.5	6
Volume equilibration	Continue	mL	3	7.5	12
Loading volume	Continue	mL	500	750	1000
Wash volume	Continue	mL	3	7.5	12
Dry time	Continue	min	5	12.5	20
Elution solvent	Categorical	/	CH_3CN	/	CH_3OH
Solvent additive	Categorical	/	0.1 % CH_2O_2	Absence	0.1 % NH_4OH
Elution volume	Continue	mL	2	7	12
Evaporation temperature	Continue	°C	30	40	50
Centrifugation	Categorical	g	0	1214	2429

Table B3. Simplex lattice mixture design that was used for optimizing the optimal composition of the solid phase eluent.

Experiment #	CH_3OH (%)	$C_5H_{12}O$ (%)	CH_3CN (%)
1	100	-	-
2	33	-	66
3	66	33	-
4	-	-	100
5	-	33	66
6	33	66	-
7	33	33	33
8	-	66	33
9	66	-	33
10	-	100	-

Table B4. Frequency table (percentage (%)) of the different SPE cartridges that were preliminary screened by eluting the target compounds by using respectively 3 mL methanol and 3 mL acetonitrile. Each cartridge was tested in replicate. The number represents the frequency of the target compound that was detected in the extract with the corresponding SPE cartridge.

	Bond Elut Plexa	Strata X	Isolute ENV+	Oasis HLB	Oasis MAX	Isolute C18	DVB Hydrophilic Speedisk	C18 Speedisk	C18 XF Speedisk	DVB Hydrophobic Speedisk
Average Phenols	**61.9**	**95.2**	**52.4**	**95.2**	**23.8**	**76.2**	**52.4**	**23.8**	**4.8**	**9.5**
2-methylphenol	66.7	100.0	33.3	100.0	0.0	100.0	100.0	0.0	0.0	0.0
4-ethylphenol	66.7	100.0	33.3	100.0	33.3	100.0	0.0	0.0	0.0	0.0
4-isopropyl phenol	66.7	100.0	33.3	100.0	33.3	100.0	0.0	0.0	0.0	0.0
4-chloro-3-methylphenol	33.3	66.7	66.7	66.7	33.3	33.3	0.0	0.0	0.0	0.0
2,5-dichloro phenol	66.7	100.0	100.0	100.0	0.0	100.0	100.0	66.7	0.0	66.7
3,4,6-trichlorophenol	66.7	100.0	0.0	100.0	0.0	0.0	66.7	0.0	0.0	0.0
Bisphenol A	66.7	100.0	100.0	100.0	66.7	100.0	100.0	100.0	33.3	0.0
Average Di-phthalates	**66.7**	**97.0**	**84.9**	**97.0**	**93.9**	**90.9**	**60.6**	**51.5**	**9.1**	**18.2**
Dimethyl phthalate	100.0	100.0	66.7	100.0	33.3	100.0	100.0	100.0	33.3	100.0
Diethyl phthalate	100.0	100.0	66.7	100.0	100.0	66.7	0.0	0.0	0.0	0.0
Dibutyl phthalate	33.3	66.7	66.7	100.0	100.0	66.7	100.0	0.0	0.0	0.0
Diamyl phthalate	66.7	100.0	100.0	100.0	100.0	100.0	66.7	66.7	0.0	0.0
Benzyl butyl phthalate	66.7	100.0	100.0	100.0	100.0	100.0	100.0	100.0	33.3	100.0
Dicyclohexyl phthalate	66.7	100.0	100.0	100.0	100.0	100.0	0.0	33.3	0.0	0.0
Dihexyl phthalate	100.0	100.0	100.0	100.0	100.0	100.0	66.7	100.0	33.3	0.0
Dibenzyl phthalate	33.3	100.0	66.7	66.7	100.0	66.7	0.0	0.0	0.0	0.0
Diethylhexyl phthalate	33.3	100.0	66.7	100.0	100.0	100.0	66.7	66.7	0.0	0.0
Dinonyl phthalate	66.7	100.0	100.0	100.0	100.0	100.0	66.7	66.7	0.0	0.0
Diisodecyl phthalate	66.7	100.0	100.0	100.0	100.0	100.0	100.0	33.3	0.0	0.0
Average Mono-phthalates	**40.7**	**92.6**	**88.9**	**96.3**	**11.1**	**51.9**	**70.4**	**88.9**	**29.6**	**33.3**
Monomethyl phthalate	33.3	100.0	100.0	100.0	0.0	33.3	100.0	66.7	33.3	33.3
Monoethyl phthalate	33.3	100.0	100.0	100.0	0.0	33.3	100.0	66.7	33.3	33.3
Monobutyl phthalate	66.7	100.0	100.0	100.0	66.7	100.0	0.0	66.7	33.3	33.3
Mono-n-pentyl phthalate	33.3	100.0	100.0	100.0	0.0	100.0	100.0	100.0	33.3	33.3
Monocyclohexyl phthalate	33.3	100.0	100.0	100.0	0.0	33.3	0.0	100.0	33.3	33.3
Monohexyl phthalate	33.3	100.0	100.0	100.0	0.0	100.0	100.0	100.0	33.3	33.3
Monobenzyl phthalate	33.3	100.0	33.3	100.0	0.0	0.0	100.0	100.0	33.3	33.3
Monoethylhexyl phthalate	66.7	66.7	66.7	100.0	0.0	33.3	33.3	100.0	0.0	33.3
Mono-isonyl phthalate	33.3	66.7	100.0	66.7	33.3	33.3	100.0	100.0	33.3	33.3

Table B5. The p-values of the F-test representing the significance of the screened parameters.

Parameters	Total	Di-phthalates	Mono-phthalates	(Alkyl)phenols
Pretreatment	0.0831	0.0097	0.1753	0.0989
pH adjustment sample	0.0359	0.0124	0.0416	0.0339
SPE-cartridge	0.0308	0.0280	0.0412	0.0214
Conditioning solvent	0.21272	0.2313	0.2469	0.1173
Volume conditioning solvent	0.2103	0.0908	0.1480	0.6537
Volume equilibration	0.0626	0.0159	0.1168	0.0836
Loading volume	0.0759	0.0085	0.3079	0.3541
Wash volume	0.1708	0.0345	0.2467	0.0739
Dry time	0.1070	0.1359	0.2059	0.0553
Elution solvent	0.0452	0.0082	0.8920	0.0415
Solvent additive	0.5070	0.0319	0.2924	0.1583
Elution volume	0.0437	0.0187	0.3090	0.0217
Evaporation temperature	0.1405	0.0854	0.5929	0.0515
Centrifugation	0.6135	0.0172	0.1777	0.1142

Table B6. Main parameter settings that were applied during untargeted data processing for extraction of features from full-scan MS data, using Compound Discoverer 2.1 software.

Parameter	Setting
Maximum retention time shift	0.2 min
Intensity threshold	2 000 000 au
m/z step size	5 ppm
Retention time width	1 min
Number of scans across a peak	7
Minimum signal to noise ratio	10

Table B7. Database of 36 di-phthalates and 15 mono-phthalates.

Compound	CAS-number	Structural formula	Molecular mass ($g.mol^{-1}$)	Exact mass (*m/z*)
2-ethyl-5-hydroxy-hexyl phthalate	40321-99-1	$C_{16}H_{22}O_5$	294.34	294.14618
Benzylbutylphthalate	85-68-7	$C_{19}H_{20}O_4$	312.36	312.13561
Bis(2-ethoxyethyl) phthalate	605-54-9	$C_{16}H_{22}O_6$	310.34	310.14109
Bis(2-methoxyethyl) phthalate	117-82-8	$C_{14}H_{18}O_6$	282.29	282.10979
Bis(2-n-butoxyethyl) phthalate	117-83-9	$C_{20}H_{30}O_6$	366.45	366.20369
Bis(4-methyl-2-pentyl) phthalate	84-63-9	$C_{20}H_{30}O_4$	334.45	334.21386
Butyl cyclohexyl phthalate	84-64-0	$C_{18}H_{24}O_4$	304.38	304.16691
Butyl decyl phthalate	89-19-0	$C_{22}H_{34}O_4$	362.50	362.24516
Di-amyl-phthalate (Di-n-pentyl phthalate)	131-18-0	$C_{18}H_{26}O_4$	306.40	306.18256
Di-iso-butyl-phthalate	84-69-5	$C_{16}H_{22}O_4$	278.34	278.15126
Di-n-butylphthalate	84-74-2	$C_{16}H_{22}O_4$	278.34	278.15126
Di-n-hexyl phthtlate	84-75-3	$C_{20}H_{30}O_4$	334.45	334.21386
Di-n-octylphthalate (Di(1-octyl) phthalate)	117-84-0	$C_{24}H_{38}O_4$	390.56	390.27646
Di-nonyl phthalate	84-76-4	$C_{26}H_{42}O_4$	418.61	418.30776
Di(2-ethylhexyl) phthalate	117-81-7	$C_{24}H_{38}O_4$	390.56	390.27646
Diallyl phthalate	131-17-9	$C_{14}H_{14}O_4$	246.26	246.08866
Dibenzyl phthalate	523-31-9	$C_{22}H_{18}O_4$	346.38	346.11996
Dicyclohexyl phthalate	84-61-7	$C_{20}H_{26}O_4$	330.42	330.18256
Diethyl phthalate	84-66-2	$C_{12}H_{14}O_4$	222.24	222.08866
Diisodecyl phthalate	26761-40-0	$C_{28}H_{46}O_4$	446.66	446.33906
Diisoheptyl phthalate	90937-19-2	$C_{22}H_{34}O_4$	362.50	362.24516
Diisohexyl phthalate	71850-09-4	$C_{20}H_{30}O_4$	334.45	334.21386
Diisononyl phthalate	68515-48-0	$C_{26}H_{42}O_4$	418.61	418.30776
Diisotridecyl phthalate	36901-61-8	$C_{34}H_{58}O_4$	530.82	530.43296
Diisoundecyl phthalate	96507-86-07	$C_{30}H_{50}O_4$	474.72	474.37036
Dimetylphthalate	131-11-3	$C_{19}H_{10}O_4$	194.18	302.05736
Dipropyl phthalate	131-16-8	$C_{14}H_{18}O_4$	250.29	250.11996
Ditridecyl phthalate	119-06-2	$C_{34}H_{58}O_2$	530.82	498.44313
Diundecyl phthalate	3648-20-2	$C_{30}H_{50}O_4$	474.72	474.37036
Diisopentyl phthalate	605-50-5	$C_{18}H_{26}O_4$	306.40	306.18256
Dioctyl tetraphthalate	6422-86-2	$C_{24}H_{38}O_4$	390.56	390.27646
Butylbenzyl phthalate	111357-64-3	$C_{19}H_{20}O_4$	312.36	312.13561
Dipropyl heptyl phthalate	53306-54-0	$C_{28}H_{46}O_4$	446.66	446.33906
Butyl octyl phthalate	84-78-6	$C_{20}H_{30}O_4$	334.45	334.21386
Diheptyl phthlate	3648-21-3	$C_{22}H_{34}O_4$	362.50	362.24516
Diisooctyl phthalate	27554-26-3	$C_{24}H_{38}O_4$	390.56	390.27646
Mono-2-ethyl-5-carboxypentyl phthalate	40809-41-4	$C_{16}H_{20}O_6$	308.33	308.12544
Mono-2-ethyl-5-oxo-hexyl phthalate	40321-98-0	$C_{16}H_{20}O_5$	292.33	292.13053
Mono-cyclohexyl phthalate	7517-36-4	$C_{14}H_{16}O_4$	248.27	248.10431
Mono-ethyl phthalate	2306-33-4	$C_{10}H_{10}O_4$	194.18	194.05736
Mono-isobutyl phthalate	308333-53-5	$C_{12}H_{14}O_4$	222.24	222.08866

Compound	CAS-number	Structural formula	Molecular mass ($g.mol^{-1}$)	Exact mass (m/z)
Mono-n-butyl phthalate	131-70-4	$C_{12}H_{14}O_4$	222.24	222.08866
Mono-n-pentyl phthalate	24539-56-8	$C_{13}H_{16}O_4$	236.26	236.10431
Mono(2-ethylhexyl) phthalate	4376-20-9	$C_{16}H_{22}O_4$	278.34	278.15126
Mono(3-carboxypropyl) phthalate	66851-46-5	$C_{12}H_{12}O_6$	252.22	252.06284
Monobenzyl phthalate	2528-16-7	$C_{15}H_{12}O_4$	256.25	256.07301
Monohexyl phthalate	24539-57-9	$C_{14}H_{18}O_4$	250.29	250.11996
Monoisopropyl phthalate	35118-50-4	$C_{11}H_{12}O_4$	208.21	208.07301
Monomethyl phthalate	4376-18-5	$C_9H_8O_4$	180.16	180.04171
Monooctyl phthalate	5393-19-1	$C_{16}H_{22}O_4$	278.34	278.15126
n-Octyl n-decyl phthalate	119-07-3	$C_{20}H_{42}O_4$	418.61	346.30776

Table B8. Comparison between the conductivity and salinity of real (Spring 2017) and reference seawater samples.

	Conductivity ($mS\ cm^{-1}$)	Salinity (PSU)
51°20'25.68"N; 3°12'12.11"O (HZ)	52.22	32.98
51°13'34.68"N; 2°56'8.00"O (HO)	49.85	31.55
51°14'48.59"N; 2°55'39.61"O (Akust39)	51.03	32.60
Reference seawater	54.78	35.11

Table B9. Detailed results of the method validation for phenols, Bisphenol A and phthalates in seawater, i.e. the recovery (n=54), limits (n=39) and repeatability (n=54) and within-laboratory reproducibility (n=18).

Compound		Recovery (%)			Limits (ng L^{-1})		Precision (%)		Linearity
	Nominal concentration (ng L^{-1})				MDL	MQL	Repeatability RSD (%)	Within-laboratory reproducibility RSD (%)	R^2
(Alkyl)phenols									
2-methyl phenol	150.0	104.1	±	7.4	75	100	7.1	11.3	0.9922
	200.0	99.7	±	1.1			1.1	4.1	
	250.0	100.2	±	2.5			2.5	2.5	
4-ethylphenol	75.0	103.1	±	8.3	25	50	8.0	8.3	0.9936
	100.0	103.3	±	1.8			1.7	2.9	
	125.0	100.3	±	0.4			0.4	3.4	
4-isopropyl phenol	300.0	103.4	±	2.4	150	200	2.3	6.0	0.9910
	400.0	104.3	±	2.6			2.5	2.0	
	500.0	99.7	±	0.2			0.2	3.3	
4-chloro-3-methylphenol	37.5	100.7	±	7.2	10	25	6.9	7.2	0.9941
	50.0	99.2	±	4.5			4.1	4.9	
	62.5	101.3	±	3.2			3.0	4.5	
2,5-dichlorophenol	75.0	107.3	±	8.4	25	50	7.9	8.6	0.9905
	100.0	103.8	±	2.2			2.1	2.7	
	125.0	99.9	±	0.6			0.6	9.7	
3,4,6-trichlorophenol	37.5	109.6	±	3.4	25	25	3.1	3.5	0.9916
	50.0	102.3	±	3.2			3.1	2.2	
	62.5	100.6	±	1.2			1.2	1.4	
Bisphenol A	75.0	102.5	±	6.2	25	50	6.1	6.6	0.9944
	100.0	101.5	±	8.3			8.1	8.7	
	125.0	101.3	±	8.8			1.3	8.8	
Di-phthalates									
Dimethyl phthalate	75.0	101.6	±	14.0	25	50	13.8	11.2	0.9912
	100.0	107.4	±	4.4			4.1	9.5	
	125.0	108.5	±	0.7			0.7	7.0	

Compound		Recovery (%)			Limits (ng L^{-1})		Precision (%)		Linearity
	Nominal concentration (ng L^{-1})				MDL	MQL	Repeatability RSD (%)	Within-laboratory reproducibility RSD (%)	R^2
Diethyl phthalate	75.0	104.6	±	8.7	25	50	8.3	10.3	0.9933
	100.0	109.8	±	10.1			9.2	9.8	
	125.0	101.1	±	5.5			5.4	6.7	
Dibutyl phthalate	15.0	101.5	±	6.8	5	10	6.7	11.5	0.9911
	20.0	95.6	±	5.2			5.4	10.2	
	25.0	105.9	±	1.8			1.7	8.0	
Diamyl phthalate	37.5	98.7	±	11.2	5.0	25	13.4	12.1	0.9961
	50.0	99.2	±	8.1			8.2	7.7	
	62.5	112.6	±	9.7			7.0	9.5	
Benzyl butyl phthalate	37.5	104.0	±	12.9	10	25	11.2	11.4	0.9968
	50.0	97.8	±	7.4			7.5	10.0	
	62.5	105.4	±	4.7			4.5	7.4	
Dicyclohexyl phtalate	30.0	103.0	±	6.2	10	20	6.0	5.8	0.9962
	40.0	102.6	±	5.5			5.3	6.7	
	50.0	101.2	±	3.9			3.8	5.9	
Dihexyl phthalate	30.0	107.2	±	11.1	10	20	10.4	11.3	0.9983
	40.0	105.0	±	4.8			4.5	5.4	
	50.0	104.1	±	6.0			5.7	6.3	
Dibenzyl phthalate	30.0	103.0	±	3.7	10	20	5.1	7.0	0.9972
	40.0	102.9	±	8.6			8.4	10.1	
	50.0	102.9	±	8.1			7.9	8.8	
Diethylheyxl phthalate	37.5	101.1	±	7.2	20	25	7.2	9.1	0.9953
	50.0	108.6	±	3.8			6.5	8.1	
	62.5	107.8	±	6.5			3.8	5.9	
Dinonyl phthalate	37.5	105.2	±	10.9	25	25	10.4	8.5	0.9952
	50.0	104.0	±	4.8			4.6	9.5	
	62.5	106.3	±	2.7			2.5	4.5	
Diisodecyl phthalate	112.5	105.2	±	6.5	50	75	6.2	10.9	0.9954
	150.0	100.5	±	1.6			1.6	8.1	

Compound	Nominal concentration (ng L^{-1})	Recovery (%)			Limits (ng L^{-1})		Precision (%)		Linearity
					MDL	MQL	Repeatibility RSD (%)	Within-laboratory reproducibility RSD (%)	R^2
	187.5	102.1	±	3.5			3.4	9.0	
Mono-phthalates									
Monomethyl phthalate	37.5	104.2	±	10.5	20	25	10.1	12.5	0.9904
	50.0	99.1	±	5.4			5.4	10.9	
	62.5	100.4	±	3.2			3.2	6.2	
Monoethyl phthalate	37.5	99.2	±	12.0	20	25	12.1	13.0	0.9943
	50.0	100.0	±	2.6			2.6	2.6	
	62.5	99.1	±	0.7			0.7	0.7	
Monobutyl phthalate	37.5	96.7	±	3.4	5	25	3.4	4.8	0.9989
	50.0	98.5	±	2.7			2.7	2.8	
	62.5	100.4	±	1.3			1.3	1.8	
Mono-n-pentyl phthalate	37.5	103.7	±	11.0	20	25	10.6	12.0	0.9914
	50.0	101.5	±	10.7			10.5	11.4	
	62.5	102.6	±	1.9			1.9	5.7	
Monocyclohexyl phthalate	15.0	99.3	±	11.5	5	10	11.6	12.3	0.9963
	20.0	99.2	±	6.6			6.7	8.9	
	25.0	99.0	±	2.7			2.8	2.6	
Monohexyl phthalate	37.5	105.4	±	7.5	20	25	7.1	12.3	0.9938
	50.0	103.4	±	4.7			4.5	6.1	
	62.5	101.2	±	3.5			3.4	3.8	
Monobenzyl phthalate	15.0	99.0	±	6.0	5	10	6.0	4.4	0.9949
	20.0	96.7	±	5.1			5.3	3.9	
	25.0	100.4	±	2.0			2.0	2.0	
Monoethylhexyl phthalate	75.0	104.0	±	6.6	25	50	7.9	6.3	0.9960
	100.0	100.7	±	4.8			4.8	3.8	
	125.0	100.6	±	3.4			3.3	3.4	
Mono-isonyl phthalate	37.5	103.5	±	4.9	20	25	4.7	9.1	0.9978
	50.0	102.4	±	2.4			2.4	5.0	
	62.5	102.0	±	1.7			1.7	3.7	

Table B10. Calibration equations for phenols, Bisphenol A and phthalates in fresh water, obtained by weighted linear regression. SD representing the standard deviation (n = 3) of the intercept and the slope, and P-value of the lack-of-fit test.

		Intercept	(SD)		Slope	(SD)		p-value	Dynamic range (ng L^{-1})
(Alkyl)phenols									
2-methyl phenol	y=	-1.00E-01	(6.24E-03)	+	3.86E-03	(2.01E-04)	x	0.9960	75-1000
4-ethylphenol	y=	6.29E-01	(4.58E-02)	+	2.09E-02	(3.06E-03)	x	0.7995	10-1000
4-isopropyl phenol	y=	-5.25E-01	(4.65E-02)	+	2.22E-02	(2.80E-03)	x	0.8830	150-1000
4-chloro-3-methylphenol	y=	8.67E-02	(1.76E-03)	+	1.30E-01	(1.31E-02)	x	0.7329	10-1000
2,5-dichlorophenol	y=	4.42E-01	(1.08E-02)	+	2.69E-02	(1.08E-03)	x	0.9990	25-1000
3,4,6-trichlorophenol	y=	4.39E-01	(6.75E-03)	+	4.55E-02	(3.71E-03)	x	0.7000	10-1000
Bisphenol A	y=	2.62E-01	(5.00E-03)	+	1.49E-02	(6.17E-04)	x	0.9970	25-1000
Di-phthalates									
Dimethyl phthalate	y=	-1.04E-01	(2.64E-03)	+	6.33E-03	(1.20E-04)	x	1.0000	25-1000
Diethyl phthalate	y=	1.07E+00	(4.08E-02)	+	4.08E-02	(2.98E-03)	x	0.8389	25-1000
Dibutyl phthalate	y=	1.06E+01	(6.16E-01)	+	1.16E-01	(3.03E-03)	x	1.0000	5-1000
Diamyl phthalate	y=	1.48E-01	(2.73E-03)	+	7.89E-02	(1.71E-03)	x	0.9500	5-1000
Benzyl butyl phthalate	y=	4.40E-01	(1.64E-02)	+	8.09E-02	(2.04E-03)	x	1.0000	10-1000
Dicyclohexyl phthalate	y=	3.79E-01	(8.50E-03)	+	3.30E-01	(6.03E-03)	x	1.0000	10-1000
Dihexyl phthalate	y=	1.88E-01	(3.11E-03)	+	5.26E-02	(9.87E-04)	x	1.0000	10-1000
Dibenzyl phthalate	y=	1.59E+00	(2.99E-02)	+	2.43E-01	(4.58E-03)	x	0.9500	10-1000
Diethylheyxl phthalate	y=	6.12E+00	(3.69E-01)	+	2.35E-01	(7.53E-03)	x	0.9990	20-1000
Dinonyl phthalate	y=	2.39E+00	(1.86E-01)	+	6.05E-02	(3.77E-03)	x	0.9670	25-1000
Diisodecyl phthalate	y=	3.25E-01	(3.90E-02)	+	3.96E-02	(5.96E-03)	x	0.9998	50-1000
Mono-phthalates									
Monomethyl phthalate	y=	-7.46E-02	(2.09E-03)	+	1.04E-02	(3.09E-04)	x	0.9974	20-1000
Monoethyl phthalate	y=	-1.04E-01	(4.18E-03)	+	6.32E-03	(2.37E-04)	x	0.9990	20-1000
Monotbutyl phthalate	y=	3.98E-01	(3.63E-02)	+	1.23E-01	(3.24E-03)	x	0.9990	5-1000
Mono-n-pentyl phthalate	y=	4.99E-02	(2.33E-03)	+	1.57E-02	(8.72E-04)	x	0.9950	20-1000
Monocyclohexyl phthalate	y=	-7.52E-01	(3.10E-02)	+	3.75E-02	(1.89E-03)	x	0.9999	5-1000
Monohexyl phthalate	y=	4.20E-02	(3.10E-03)	+	7.63E-03	(3.98E-04)	x	0.9880	20-1000
Monobenzyl phthalate	y=	-8.15E-01	(4.00E-02)	+	4.17E-02	(2.32E-03)	x	0.8569	5-1000
Monoethylhexyl phthalate	y=	1.40E-01	(9.75E-03)	+	5.84E-03	(4.44E-04)	x	0.8810	25-1000
Mono-isonyl phthalate	y=	1.40E-01	(1.17E-02)	+	5.85E-03	(3.24E-04)	x	0.6426	20-1000

Table B11. Calibration equations for the target APs and PAEs in fresh water, obtained by weighted linear regression. SD representing the standard deviation (n = 3) of the intercept and the slope, and P-value of the lack-of-fit test.

		Intercept	(SD)		Slope	(SD)		p-value	Dynamic range (ng L^{-1})
(Alkyl)phenols									
2-methyl phenol	y=	2.18E-01	(1.13E-02)	+	2.72E-03	(1.01E-02)	x	0.9761	50-1000
4-ethylphenol	y=	8.02E-02	(5.02E-03)	+	5.48E-03	(1.11E-02)	x	0.7116	25-1000
4-isopropyl phenol	y=	-5.35E-02	(4.21E-03)	+	4.71E-03	(6.26E-03)	x	0.7329	150-1000
4-chloro-3-methylphenol	y=	-4.69E-01	(8.46E-03)	+	2.89E-02	(4.24E-02)	x	0.7256	20-1000
2,5-dichlorophenol	y=	-2.32E-01	(5.51E-03)	+	8.31E-03	(7.79E-03)	x	0.8292	25-1000
3,4,6-trichlorophenol	y=	6.13E-01	(8.48E-03)	+	2.26E-02	(4.60E-02)	x	0.6300	20-1000
Bisphenol A	y=	-1.18E-01	(1.92E-03)	+	2.72E-03	(4.87E-03)	x	0.9172	10-1000
Di-phthalates									
Dimethyl phthalate	y=	1.63E-02	(3.93E-04)	+	6.14E-03	(2.84E-04)	x	0.9400	25-1000
Diethyl phthalate	y=	5.82E-01	(2.09E-02)	+	3.69E-02	(4.21E-02)	x	0.8221	10-1000
Dibutyl phthalate	y=	4.63E+00	(2.62E-01)	+	5.27E-02	(1.12E-01)	x	0.8400	5-1000
Diamyl phthalate	y=	2.46E-02	(4.20E-04)	+	3.88E-02	(4.96E-04)	x	0.8075	5-1000
Benzyl butyl phthalate	y=	1.30E-01	(4.41E-03)	+	2.83E-02	(3.23E-03)	x	0.9600	5-1000
Dicyclohexyl phthalate	y=	5.49E-02	(1.12E-03)	+	1.43E-01	(9.82E-04)	x	0.8400	10-1000
Dihexyl phthalate	y=	-2.51E-01	(3.86E-03)	+	2.98E-02	(4.53E-03)	x	0.8700	10-1000
Dibenzyl phthalate	y=	-7.38E-01	(1.37E-02)	+	8.24E-02	(1.28E-02)	x	0.8360	10-1000
Diethylheyxl phthalate	y=	1.16E+00	(6.91E-02)	+	1.09E-01	(3.44E-02)	x	0.9091	20-1000
Dinonyl phthalate	y=	2.78E-01	(1.94E-02)	+	2.04E-02	(1.68E-02)	x	0.9573	25-1000
Diisodecyl phthalate	y=	2.23E-02	(2.24E-03)	+	1.91E-02	(3.32E-03)	x	0.9798	25-1000
Mono-phthalates									
Monomethyl phthalate	y=	2.91E-03	(6.94E-05)	+	3.64E-05	(8.03E-05)	x	0.9874	25-1000
Monoethyl phthalate	y=	1.63E-02	(5.50E-04)	+	5.41E-03	(5.44E-04)	x	0.9091	20-1000
Monotbutyl phthalate	y=	1.68E-01	(1.50E-02)	+	2.23E-02	(4.38E-03)	x	0.9291	10-1000
Mono-n-pentyl phthalate	y=	1.26E-02	(5.00E-04)	+	4.60E-02	(5.80E-04)	x	0.9055	25-1000
Monocyclohexyl phthalate	y=	-1.46E+00	(5.41E-02)	+	8.51E-02	(6.30E-02)	x	0.8899	5-1000
Monohexyl phthalate	y=	1.25E-02	(8.45E-04)	+	1.20E-02	(5.98E-04)	x	0.8694	20-1000
Monobenzyl phthalate	y=	-1.11E+00	(5.28E-02)	+	7.88E-02	(5.69E-02)	x	0.7198	5-1000
Monoethylhexyl phthalate	y=	-2.16E-01	(1.43E-02)	+	8.15E-03	(1.54E-02)	x	0.7577	25-1000
Mono-isonyl phthalate	y=	-6.63E-02	(5.23E-03)	+	3.76E-03	(3.24E-03)	x	0.5334	25-1000

Table B12. Summary of results for the cross-validation in freshwater, i.e. the recovery (n=18), limits (n=18) and repeatability (n=18) and within-laboratory reproducibility (n=18).

Compound	Recovery (%)	Limits		Precision (%)	Linearity
		MDL (ng L^{-1})	MQL (ng L^{-1})	Repeatability RSD (%)	R^2
(Alkyl)phenols					
2-methyl phenol	105.2 ± 14.7	50	100	14.0	0.9921
4-ethylphenol	104.2 ± 7.4	25	50	7.1	0.9940
4-isopropyl phenol	100.9 ± 8.8	150	200	8.8	0.9928
4-chloro-3-methylphenol	102.3 ± 3.4	20	25	3.1	0.9978
2,5-dichlorophenol	102.5 ± 2.7	25	25	2.7	0.9999
3,4,6-trichlorophenol	109.0 ± 4.0	20	25	3.7	0.9971
Bisphenol A	100.7 ± 10.7	10	25	10.9	0.9921
Di-phthalates					
Dimethyl phthalate	101.1 ± 13.3	25	50	13.2	0.9925
Diethyl phthalate	107.1 ± 8.1	10	25	7.6	0.9905
Dibutyl phthalate	95.1 ± 11.2	5.0	10.0	11.8	0.9961
Diamyl phthalate	102.1 ± 13.1	5	25	12.8	0.9930
Benzyl butyl phthalate	109.1 ± 11.6	5	25	10.6	0.9976
Dicyclohexyl phthalate	99.2 ± 11.7	10	20	1.7	0.9995
Dihexyl phthalate	106.1 ± 12.2	10	20	11.5	0.9961
Dibenzyl phthalate	103.7 ± 2.2	10	25	2.6	0.9995
Diethylheyxl phthalate	97.2 ± 7.2	20	25	9.2	0.9990
Dinonyl phthalate	106.9 ± 8.4	25	50	7.8	0.9947
Diisodecyl phthalate	107.1 ± 5.7	25	50	5.3	0.9997
Mono-phthalates					
Monomethyl phthalate	102.9 ± 8.4	25	50	8.2	0.9964
Monoethyl phthalate	99.3 ± 7.9	20	25	7.9	0.9905
Monotbutyl phthalate	99.0 ± 2.7	10	25	2.8	0.9992
Mono-n-pentyl phthalate	100.1 ± 13.7	25	50	13.7	0.9924
Monocyclohexyl phthalate	98.8 ± 3.4	5	10	3.4	0.9947
Monohexyl phthalate	101.2 ± 14.7	20	25	14.5	0.9961
Monobenzyl phthalate	100.4 ± 4.7	5	10	4.7	0.9971
Monoethylhexyl phthalate	103.8 ± 5.4	25	50	5.2	0.9912
Mono-isonyl phthalate	104.0 ± 8.8	25	25	8.4	0.9966

Table B13. The tentatively identified unknowns that are assigned to the backbone of plasticizers, with m/z 167.033, 149.023, 121.029 and 65.039 as characteristic fragments for the phthalates. ID represents the identifier or the unknown compound.

Type of plasticizer	ID	Molecular mass ($g.mol^{-1}$)	t_r (min)	Assignment
(Alkyl)phenols	9	472.216	5.75	$[M-H-CH_4]^-$
	14	394.239	6.27	$[M-H-CH_4]^-$
	16	460.144	5.40	$[M-H-CH_4]^-$
	54	200.050	2.37	$[M-H-CH_4]^-$, $[M-H-CH_3]^-$
	84	401.180	5.34	$[M-H-CH_4]^-$
	87	461.143	5.45	$[M-H-CH_4]^-$
	95	325.200	5.23	$[M-H-CH_4]^-$
	106	532.183	5.82	$[M-H-CH_4]^-$
	113	473.217	5.93	$[M-H-CH_4]^-$
	194	211.938	0.67	$[M-H-HCl]^-$
	344	150.067	2.04	$[M-H-CH_3]^-$
	409	350.231	5.80	$[M-H-CH_4]^-$
	445	509.194	5.78	$[M-H-HCl]^-$
	485	395.242	6.38	$[M-H-CH_4]^-$
	540	508.193	7.55	$[M-H-HCl]^-$
	619	201.054	2.38	$[M-H-CH_3]^-$
	818	888.578	9.44	$[M-H-HCl]^-$
	911	245.226	7.15	$[M-H-CH_4]^-$
	959	289.916	1.58	$[M-H-HCl]^-$
	1002	107.047	7.55	$[M-H-CH_4]^-$
Phthalates	20	278.152	5.22	All characteristic fragments
	38	398.241	5.44	All characteristic fragments
	51	197.120	4.97	All characteristic fragments
	79	390.277	7.02	All characteristic fragments
	86	398.241	5.68	All characteristic fragments
	117	309.170	4.77	All characteristic fragments
	142	418.308	6.95	All characteristic fragments
	143	278.152	5.66	All characteristic fragments
	159	390.277	6.68	All characteristic fragments
	188	148.110	2.74	All characteristic fragments, except 167.033
	200	166.006	1.21	All characteristic fragments
	242	148.075	9.81	All characteristic fragments, except 167.033
	301	197.120	5.12	All characteristic fragments
	334	279.155	5.33	All characteristic fragments
	342	148.016	5.25	All characteristic fragments, except 167.033
	446	277.896	3.63	All characteristic fragments
	454	330.202	4.43	All characteristic fragments
	484	206.874	5.19	All characteristic fragments
	615	191.131	6.62	All characteristic fragments
	659	239.085	4.32	All characteristic fragments
	665	183.162	4.74	All characteristic fragments
	670	391.280	6.47	All characteristic fragments
	759	154.063	4.48	All characteristic fragments, except 167.033
	879	222.166	4.82	All characteristic fragments
	968	148.016	1.86	All characteristic fragments, except 167.033

APPENDIX C – HYDROPHILIC DIVINYLBENZENE FOR EQUILIBRIUM SORPTION OF EMERGING ORGANIC CONTAMINANTS IN AQUATIC MATRICES

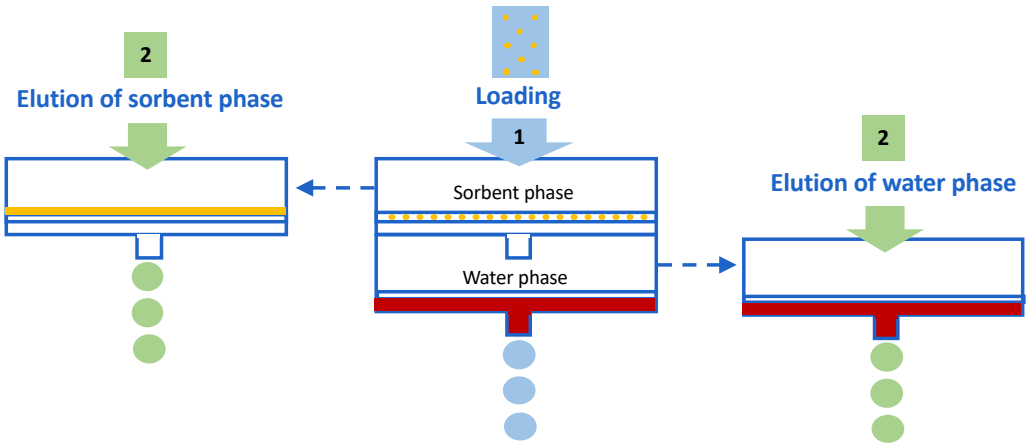
Sorbent and water analysis

Details regarding to the sample analysis (SPE-LC-Orbitrap HRMS) have been published earlier (Huysman et al., 2019, 2017; Vanryckeghem et al., 2019), and here only the main differences are briefly described:

i) Pharmaceuticals, pesticides and personal care products were extracted with Oasis® HLB cartridges (6cc, 200mg). Elution was performed with 5 mL of CH_3OH/CH_3CN (50/50, v/v %). Chromatographic separation was achieved by UHPLC using CH_3OH and H_2O both acidified with 0.1% and 0.01% formic acid for the positive and negative ionisation mode, respectively. Ionisation was performed using a heated electrospray ionisation (HESI) source operating in full-scan mode for both modes.

ii) Steroidal EDCs were extracted by using hydrophilic DVB cartridges. Elution was performed using sequential 5 mL of pure CH_3CN and 5 mL of CH_3CN, with the latter being acidified with 0.1% formic acid. The analytes were separated by UHPLC using CH_3OH and H_2O, both containing with 0.1 % formic acid. The ionization was realised by heated electrospray chemical ionization, and determined by full-scan events in positive and negative ionization mode.

iii) Phthalates and alkylphenols were extracted using Oasis® HLB cartridges (6cc, 500mg). Elution was executed by using 9 mL of 0.1% formic acid in CH_3CN. The analytes were chromatographically separated using CH_3CN/H_2O, both containing 0.1 % NH_4OH. The ionization was realised by heated electrospray ionization, and determined by full-scan events in both the positive and negative ion mode.



	Steroidal EDCs	Pharmaceuticals, personal care products & pesticides	(alkyl)phenols and phthalates
1. Loading of sample	500 mL	200 mL	500 mL
2. Elution of both phases:	5 mL of pure CH_3CN & 5 mL of CH_3CN with 0.1% formic acid	5 mL of CH_3OH/CH_3CN	9 mL of 0.1% formic acid in CH_3CN
SPE-cartridge:	Bakerbond Speedisk®	Oasis HLB (200mg)	Oasis HLB (500mg)

Figure C1. Schematic overview of the sorbent and water analysis. During step 1 a glass fibre filter was used, in step 2 the most suitable cartridge (red colored) for each group of organic contaminants was used (see specifications in table under the schematic overview). The yellow dots and yellow colored area represent the hydrophilic DVB sorbent that was used in the experiments.

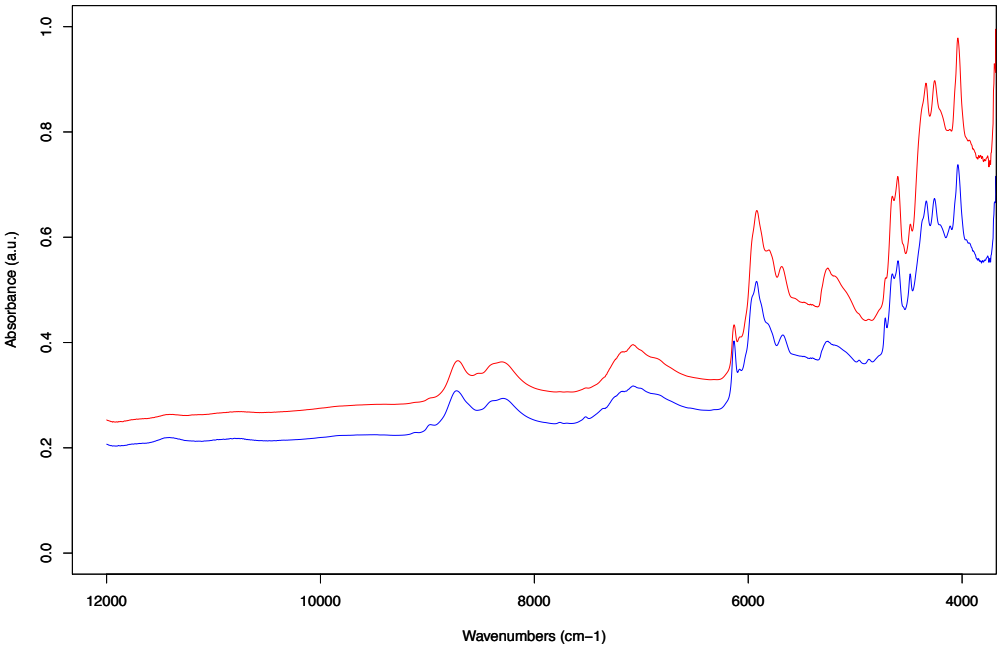


Figure C2. The averaged NIR spectra (n=3) of hydrophilic DVB (blue) and Oasis™ HLB(red).

Table C1. The average mass balance (%) and the corresponding analytical repeatability (% RSDs) of each organic contaminants studied.

Compound	Mass balance		Repeatability	Number of
	Average (%)	SD (%)	(% RSD)	datapoints
(alkyl)phenols				
2,5-dichlorophenol	78	2	3	8
bisphenol A	85	5	5	8
isopropylphenol	76	5	6	5
trichlorophenol	76	4	5	8
personal care products				
DEET	52	7	14	7
ethylparaben	42	4	9	6
methylparaben	44	5	10	3
piperonylbutoxide	115	9	8	6
propylparaben	49	6	11	7
pesticides				
acetamiprid	49	8	16	8
alachlor	60	4	6	6
atrazine	57	3	6	7
chlorfenvinphos	63	3	5	8
chloridazon	43	8	19	8
clothianidin	47	6	13	8
dichlorophenoxyacetic acid	62	2	4	8
dimethoate	46	6	13	7
dinoseb	79	12	15	6
diuron	47	5	10	7
flufenacet	57	4	6	7
imidacloprid	42	8	18	8
irgarol	58	3	6	6
isoproturon	52	6	12	7
linuron	47	5	10	6
mecoprop	64	2	3	8
methiocarb	55	4	6	6
metolachlor	58	3	6	6
pirimicarb	50	3	5	7
quinoxyfen	113	10	8	5
simazine	47	4	9	7
terbuthylazine	51	2	5	5
terbutryn	57	2	3	6
thiacloprid	53	5	10	4
thiamethoxam	49	3	6	5
pharmaceuticals				
alprazolam	65	7	10	8
amitriptyline	57	3	6	6
azithromycin	63	7	11	5
bezafibrate	58	4	7	8
bisoprolol	56	6	11	8
carbamazepine	51	6	11	8
chloramphenicol	55	8	14	8
chlorfibric acid	59	2	3	8
clarithromycin	73	10	14	8
diazepam	60	4	6	7
diclofenac	68	5	7	7
efavirenz	47	5	10	6
enrofloxacin	65	2	4	8
flumequine	65	1	2	8
fluoxetine	53	3	5	6
ifosfamide	54	4	7	8
indomethacin	47	7	16	7
ketoprofen	57	3	6	7

Compound	Mass balance		Repeatability	Number of
	Average (%)	SD (%)	(% RSD)	datapoints
metoprolol	54	7	13	8
moxifloxacin	79	5	7	2
nalidixic acid	64	2	3	8
nevirapine	49	10	20	8
oseltamivir ethylester	61	5	8	8
paroxetine	49	4	8	6
propranolol	51	5	10	8
rimantadine	57	4	7	7
sarafloxacin	65	2	3	7
sulfadoxin	58	1	2	6
sulfamethazine	57	3	6	7
sulfamethoxazole	62	4	6	6
tetracycline	67	5	7	2
trimethoprim	52	7	14	6
phthalates				
benzylbutyl phthalate	82	4	5	7
diamyl phthalate	83	5	7	7
dibutyl phthalate	70	2	3	7
dicyclohexyl phthalate	67	3	5	6
diethyl phthalate	72	3	5	8
diethylheyl phthalate	92	2	2	3
dihexyl phthalate	71	3	5	3
dimethyl phthalate	78	4	6	8
dinonyl phthalate	80	7	8	6
monobenzyl phthalate	85	1	1	8
monocyclohexyl phthalate	80	4	5	8
monoethyl phthalate	88	8	9	7
monohexyl phthalate	83	4	5	8
monomethylphthalate	85	7	8	6
mono-n-pentyl phthalate	76	5	7	8
steroidal EDCs				
1,4-androstadienedione	78	13	17	8
11-ketoetiocholanolone	90	17	19	6
11-ketotestosterone	73	8	11	7
5α-dihydrotestosterone	71	10	14	7
11β-hydroxyandrosterone	96	18	19	8
17α-acetoxyprogesterone	77	5	7	8
17α-hydroxyprogesterone	72	8	11	8
17α-testosterone	88	3	4	7
17α-trenbolone	83	13	16	7
17β-testosterone	67	5	8	7
17β-trenbolone	70	15	21	7
19-Norethindron	69	4	6	8
19-nortestosterone	72	3	4	8
α-zearalenol	79	4	5	7
α-zeranol	65	1	2	2
β-zearalenol	71	3	4	5
β-zeranol	72	10	14	6
androstenedione	88	17	20	8
androsterone	77	9	12	6
caproxyprogesterone	70	4	6	6
chlorotestosteron acetate	72	10	14	6
cortisol	82	7	9	6
cortisone	94	11	12	8
dexamethasone	80	5	6	5
dienoestrol	91	6	6	8
diethylstilbestrol	72	12	17	6
epi-androsterone	73	11	15	6
estrone	60	8	14	8

Compound	Mass balance		Repeatability	Number of
	Average (%)	SD (%)	(% RSD)	datapoints
ethynyl testosterone	72	2	2	6
flugestone acetate	65	7	10	7
fluoxymesterone	89	7	8	6
gestodene	65	3	5	5
medroxyprogesterone	65	3	5	5
medroxyprogesterone acetate	60	2	4	6
megestrol	70	6	8	7
megestrol acetate	68	6	8	6
mestanolone	88	8	9	7
methylboldenone	67	2	3	7
methylprogesterone	65	5	7	6
norethandrolone	65	3	5	5
norgestrel	65	5	7	6
prednisolone	73	8	10	8
prednisone	88	9	10	8
progesterone	65	5	7	4
stanozolol	80	7	9	6
testosterone phenylpropionate	75	8	10	7
testosterone acetate	72	6	8	6
testosterone benzoate	72	8	11	7
testosterone propionate	67	11	16	6
trenbolone acetate	82	8	10	7

Table C2. The calculated sorbent-water equilibrium partitioning coefficients (log K_{sw}) with their corresponding standard deviations (SD). n represents the number of datapoints originating from time points after which equilibrium was established. Log P represents the polarity index of the compound.

Compound	Log K_{sw}		n	Log P
	Average	SD		
(alkyl)phenols				
2,5-dichlorophenol	4.91	0.76	4	3.03
bisphenol A	5.82	0.35	4	1.56
isopropylphenol	5.32	0.22	4	2.99
trichlorophenol	4.14	0.61	4	3.69
personal care products				
DEET	5.87	0.42	4	2.02
ethylparaben	5.93	0.37	5	2.47
methylparaben	5.53	0.23	4	1.96
piperonylbutoxide	5.95	0.17	2	4.75
propylparaben	5.95	0.80	2	3.04
pesticides				
acetamiprid	5.55	0.12	5	0.80
alachlor	5.69	0.80	3	3.52
atrazine	6.09	0.26	6	2.61
chlorfenvinphos	6.03	0.51	5	3.81
chloridazon	5.10	0.15	4	1.14
clothianidin	5.08	0.10	4	0.70
dichlorophenoxyacetic acid	4.89	0.83	2	2.81
dimethoate	5.10	0.12	5	0.78
dinoseb	6.01	0.48	4	3.56
diuron	6.48	0.16	4	2.68
flufenacet	5.92	0.14	6	3.20
imidacloprid	5.38	0.17	6	0.57
irgarol	6.31	0.24	6	3.26
isoproturon	6.16	0.24	6	2.89
linuron	6.63	0.49	4	3.20
mecoprop	4.69	0.12	5	3.13
methiocarb	5.06	0.27	2	2.92
metolachlor	5.95	0.20	6	3.13
pirimicarb	5.73	0.18	6	1.70
quinoxyfen	5.74	-	1	4.66
simazine	5.91	0.25	5	2.18
terbuthylazine	6.93	0.27	6	3.40
terbutryn	6.18	0.62	5	3.74
thiacloprid	6.08	0.20	6	1.26
thiamethoxam	4.89	0.11	5	- 0.13
pharmaceuticals				
alprazolam	5.53	0.25	5	2.12
amitriptyline	6.20	0.17	5	4.92
azithromycin	4.56	0.26	6	4.02
bezafibrate	5.49	0.08	7	2.50
bisoprolol	5.27	0.24	6	1.87
carbamazepine	6.15	0.17	6	2.45
chloramphenicol	5.42	0.12	5	1.14
clorfibric acid	4.27	0.06	4	2.43
clarithromycin	4.90	0.25	6	3.16
diazepam	6.03	0.14	5	2.82
diclofenac	6.06	0.14	2	4.51
efavirenz	6.00	0.24	5	4.60
enrofloxacin	5.74	0.40	3	2.31
flumequine	4.36	0.13	5	1.60
fluoxetine	4.02	0.09	5	4.05
ifosfamide	6.10	0.14	6	0.86

Compound	Log K_{sw}		n	Log P
	Average	SD		
indomethacin	4.88	0.10	6	4.27
ketoprofen	5.37	0.07	6	3.12
metoprolol	5.38	0.19	6	1.88
moxifloxacin	4.35	0.21	5	2.90
nalidixic acid	3.81	0.09	6	1.59
nevirapine	5.69	0.19	6	2.50
oseltamivir ethylester	4.88	0.03	6	1.71
paroxetine	6.25	0.37	6	3.60
propranolol	6.17	0.19	6	3.48
rimantadine	5.19	0.09	6	3.60
sarafloxacin	4.20	0.13	5	1.07
sulfadoxin	4.14	0.19	5	0.70
sulfamethazine	4.66	0.12	5	0.89
sulfamethoxazole	3.96	0.38	5	0.89
tetracycline	3.91	0.30	6	-1.30
trimethoprim	5.69	0.17	6	0.91
phthalates				
benzylbutyl phthalate	5.43	0.55	4	4.91
diamyl phthalate	5.36	0.07	4	5.77
dibutyl phthalate	5.41	0.40	4	4.75
dicyclohexyl phthalate	5.54	0.74	4	6.64
diethyl phthalate	5.46	0.56	5	2.71
diethylheyl phthalate	5.15	0.39	3	8.52
dihexyl phthalate	5.55	0.97	4	6.79
dimethyl phthalate	5.62	0.27	6	1.70
dinonyl phthalate	4.65	-	1	9.85
monobenzyl phthalate	6.67	0.35	5	2.82
monocyclohexyl phthalate	6.44	0.36	3	3.10
monoethyl phthalate	5.60	0.22	4	1.64
monohexyl phthalate	5.70	0.19	2	3.68
monomethylphthalate	5.72	0.61	4	1.13
mono-n-pentyl phthalate	6.23	0.61	3	3.17
steroidal EDCs				
1,4-androstadienedione	5.48	0.18	4	2.62
11-ketoetiocholanolone	6.38	0.06	2	1.30
11-ketotestosterone	5.51	0.32	5	1.30
5α-dihydrotestosterone	5.95	0.08	2	1.79
11β-hydroxyandrosterone	5.80	0.66	4	1.97
17α-acetoxyprogesterone	5.38	0.15	4	3.64
17α-hydroxyprogesterone	5.73	0.61	4	3.64
17α-testosterone	6.86	1.14	2	3.18
17α-trenbolone	5.49	0.16	4	2.32
17β-testosterone	5.22	0.07	2	3.18
17β-trenbolone	5.39	0.21	4	2.32
19-norethindron	5.96	0.87	3	2.89
19-nortestosterone	5.72	0.28	6	2.90
α-zearalenol	5.43	0.18	4	4.17
α-zeranol	5.86	0.44	4	3.09
β-zearalenol	5.77	-	1	4.18
β-zeranol	5.62	0.48	5	3.09
androstenedione	6.38	0.06	4	2.72
androsterone	5.57	0.36	4	3.93
caproxyprogesterone	5.89	0.36	4	5.68
chlorotestosteron acetate	6.13	0.51	4	4.61
cortisol	5.32	0.64	4	1.76
cortisone	4.97	0.01	2	1.43
dexamethasone	5.56	0.34	4	2.03
diethylstilbestrol	6.03	-	1	5.33
epi-androsterone	6.24	0.15	2	3.93

Compound	Log K_{sw}		n	Log P
	Average	SD		
estrone	6.39	0.53	2	3.62
ethynyl testosterone	6.18	0.10	2	3.14
flugestone acetate	6.26	0.31	2	2.82
fluoxymesterone	5.63	0.53	2	2.27
gestodene	5.82	0.41	5	2.02
medroxyprogesterone	5.62	0.29	4	3.58
medroxyprogesterone acetate	5.64	0.34	4	4.17
megestrol	5.51	0.32	3	3.23
megestrol acetate	5.92	0.86	4	3.75
mestanolone	5.89	0.61	4	4.13
methylboldenone	5.62	0.24	3	3.47
methylprogesterone	5.62	0.29	4	5.21
norethandrolone	6.09	1.14	4	3.78
norgestrel	5.60	0.09	4	3.37
prednisolone	6.02	0.22	2	1.64
prednisone	5.81	-	1	1.57
progesterone	5.84	0.58	4	3.83
stanozolol	5.96	0.34	5	5.41
testosterone phenylpropionate	6.34	-	1	6.29
testosterone acetate	5.66	0.42	4	3.14
testosterone benzoate	6.52	0.54	3	6.00
testosterone propionate	6.01	0.52	3	4.39
trenbolone acetate	4.97	-	1	4.02

Table C3. The calculated Freundlich coefficients (K_F) and exponents (n) with their corresponding standard deviation (SD) and significance (p-value). The p-value represents the goodness-of-fit through the Freundlich model. The Asterix(*) corresponds to compounds that could not be calculated, because concentrations were detected below detection limits, resulting in in lack-of data points for constructing a Freundlich model. T, pH and S corresponds to temperature, acidity and salinity, respectively.

		Freundlich coefficient			Freundlich exponent (n)		
Compound	Model p-value	K_F	SD	p-value	n	SD	p-value
(alkyl)phenols							
2,5-dichlorophenol*							
bisphenol A	0.11852	2.72E+04	4.05E+00	0.00010	0.27	0.14	0.118515
isopropylphenol	0.01153	1.29E+03	2.04E+00	0.00011	0.54	0.14	0.011526
trichlorophenol	0.00127	2.43E+01	1.21E+00	0.01456	0.77	0.12	0.001273
personal care products							
DEET	0.00043	3.95E+03	1.33E+00	0.00000	0.62	0.08	0.000429
ethylparaben	0.00030	6.54E+03	8.31E-01	0.00000	0.49	0.04	0.000301
methylparaben	0.00012	2.65E+03	9.37E-01	0.00000	0.51	0.05	0.000123
piperonylbutoxide	0.28958	2.88E+04	5.28E+00	0.07301	0.44	0.22	0.289584
propylparaben	0.02972	3.01E+04	4.31E-01	0.00594	0.37	0.02	0.029716
pesticides							
acetamiprid	0.00023	2.16E+03	1.12E+00	0.00000	0.54	0.06	0.000228
alachlor	0.08505	5.33E+03	3.85E+00	0.01415	0.59	0.18	0.085049
atrazine	0.00055	4.58E+03	1.31E+00	0.00000	0.60	0.08	0.000552
chlorfenvinphos	0.08138	2.16E+04	3.20E+00	0.00541	0.48	0.14	0.081379
chloridazon	0.00019	1.42E+03	1.04E+00	0.00000	0.52	0.05	0.000192
clothianidin	0.00016	1.10E+03	1.03E+00	0.00000	0.52	0.05	0.000161
dichlorophenoxyacetic acid	0.00164	5.55E+02	1.57E+00	0.00010	0.49	0.08	0.001643
dimethoate	0.00051	1.16E+03	1.33E+00	0.00002	0.54	0.07	0.000512
dinoseb	0.00468	1.64E+04	1.30E+00	0.00007	0.43	0.06	0.004682
diuron	0.00013	9.17E+03	8.10E-01	0.00000	0.52	0.05	0.000134
flufecet	0.00169	3.42E+03	2.04E+00	0.00003	0.66	0.11	0.001688
imidacloprid	0.00017	1.92E+03	1.12E+00	0.00000	0.54	0.05	0.000172
irgarol	0.00283	4.91E+03	1.93E+00	0.00002	0.71	0.13	0.002832
isoproturon	0.00075	5.42E+03	1.35E+00	0.00000	0.57	0.08	0.000753
linuron	0.01539	2.86E+04	1.66E+00	0.00011	0.44	0.09	0.015391
mecoprop	0.00131	6.84E+02	1.68E+00	0.00011	0.55	0.08	0.001307
methiocarb	0.22513	3.51E+04	3.21E+00	0.04294	0.41	0.15	0.225128
metolachlor	0.00145	3.23E+03	1.90E+00	0.00002	0.67	0.11	0.001454
pirimicarb	0.00043	1.74E+03	1.51E+00	0.00002	0.72	0.09	0.000430
quinoxyfen*							
simazine	0.00018	3.75E+03	1.04E+00	0.00000	0.56	0.06	0.000180
terbuthylazine	0.00076	6.87E+03	1.35E+00	0.00000	0.65	0.09	0.000756
terbutryn	0.01763	7.25E+03	2.60E+00	0.00019	0.64	0.16	0.017627
thiacloprid	0.00012	5.03E+03	9.52E-01	0.00000	0.55	0.05	0.000122
thiamethoxam	0.00088	9.72E+02	1.50E+00	0.00004	0.51	0.07	0.000881
pharmaceuticals							
alprazolam	0.00069	8.57E+02	1.64E+00	0.00007	0.73	0.10	0.000685
amitriptyline	0.00280	3.90E+03	2.05E+00	0.00003	0.71	0.13	0.002799
azithromycin	0.00002	6.95E+01	8.03E-01	0.00019	0.87	0.05	0.000017
bezafibrate	0.00058	1.66E+03	1.73E+00	0.00004	0.65	0.08	0.000582
bisoprolol	0.00015	4.30E+02	1.38E+00	0.00008	0.80	0.08	0.000151
carbamazepine	0.00021	4.81E+03	1.06E+00	0.00000	0.58	0.06	0.000212
chloramphenicol	0.00006	1.43E+03	9.72E-01	0.00000	0.61	0.05	0.000061
clofibric acid	0.00110	2.62E+02	1.41E+00	0.00021	0.55	0.08	0.001100
clarithromycin	0.00013	3.25E+02	1.43E+00	0.00016	0.76	0.07	0.000128
diazepam	0.00187	2.16E+03	1.95E+00	0.00005	0.72	0.12	0.001869

		Freundlich coefficient			Freundlich exponent (n)		
Compound	**Model p-value**	**K_F**	**SD**	**p-value**	**n**	**SD**	**p-value**
diclofenac	0.00126	1.08E+04	1.48E+00	0.00000	0.43	0.07	0.001264
efavirenz*							
enrofloxacin	0.00002	1.12E+02	7.23E-01	0.00004	0.73	0.05	0.000018
flumequine	0.00007	9.90E+01	7.66E-01	0.00007	0.64	0.05	0.000070
fluoxetine	0.00143	2.84E+03	1.95E+00	0.00003	0.74	0.12	0.001435
ifosfamide	0.00035	6.80E+02	1.34E+00	0.00004	0.60	0.07	0.000351
indomethacin	0.01271	7.27E+03	2.22E+00	0.00010	0.46	0.11	0.012708
ketoprofen	0.00055	1.57E+03	1.50E+00	0.00002	0.60	0.08	0.000545
metoprolol	0.00021	8.34E+02	1.27E+00	0.00002	0.69	0.07	0.000208
moxifloxacin	0.00002	1.26E+02	7.42E-01	0.00004	0.70	0.05	0.000022
nalidixic acid*							
nevirapine	0.00028	1.61E+03	1.32E+00	0.00001	0.67	0.07	0.000276
oseltamivir ethylester	0.00007	2.67E+02	1.14E+00	0.00008	0.78	0.07	0.000070
paroxetine	0.00325	5.99E+03	2.30E+00	0.00003	0.70	0.13	0.003245
propranolol	0.00095	3.62E+03	1.64E+00	0.00001	0.68	0.10	0.000950
rimantadine	0.00005	6.43E+02	8.68E-01	0.00000	0.67	0.05	0.000049
sarafloxacin	0.00002	1.49E+02	7.25E-01	0.00002	0.68	0.04	0.000017
sulfadoxin	0.01135	6.11E+02	2.25E+00	0.00051	0.42	0.11	0.011352
sulfamethazine	0.00091	8.52E+02	1.57E+00	0.00006	0.53	0.08	0.000913
sulfamethoxazole	0.01530	1.77E+02	1.71E+00	0.00105	0.40	0.11	0.015299
tetracycline	0.00012	4.83E+00	5.21E-01	0.09322	1.04	0.10	0.000124
trimethoprim	0.00028	1.62E+03	1.13E+00	0.00000	0.62	0.07	0.000280
phthalates							
benzylbutyl phthalate	0.03349	4.17E+03	4.47E+00	0.00109	0.46	0.16	0.033486
diamylphthalate	0.07303	5.54E+03	4.83E+00	0.00114	0.41	0.18	0.073032
dibutyl phthalate	0.00128	4.86E+03	1.86E+00	0.00001	0.44	0.07	0.001284
dicyclohexylphthalate	0.14100	6.02E+03	5.98E+00	0.00270	0.37	0.21	0.141002
diethyl phthalate	0.23295	1.77E+03	7.64E+00	0.02451	0.60	0.45	0.232949
diethylhexyl phthalate	0.19759	3.70E+00	3.47E+00	0.83908	1.20	0.81	0.197588
dihexyl phthalate	0.06720	4.16E+03	5.21E+00	0.00217	0.43	0.18	0.067198
dimethyl phthalate	0.00652	1.61E+03	2.45E+00	0.00020	0.62	0.14	0.006519
dinonyl phthalate	0.01965	3.99E-02	-5.31E+00	0.43501	1.55	0.46	0.019645
monobenzyl phthalate	0.00559	1.25E+03	6.02E+00	0.01446	1.15	0.25	0.005588
monocyclohexyl phthalate	0.79206	1.35E+04	3.71E+00	0.00045	0.04	0.14	0.792062
monoethyl phthalate	0.00866	2.09E+03	2.42E+00	0.00014	0.55	0.13	0.008658
monohexyl phthalate	0.78958	5.74E+04	3.85E+00	0.00087	0.03	0.11	0.789581
monomethyl phthalate	0.00569	3.20E+03	2.01E+00	0.00015	0.50	0.09	0.005688
mono-n-pentyl phthalate	0.91805	3.23E+04	4.12E+00	0.00034	0.01	0.14	0.918054
steroidal EDCs							
1,4-androstadienedione	0.03303	1.51E+02	3.01E+00	0.02214	1.62	0.51	0.033025
11-ketoetiocholanolone	0.00229	7.28E+01	1.29E+00	0.00343	2.52	0.36	0.002289
11-ketotestosterone	0.00249	4.88E+01	1.41E+00	0.00954	1.89	0.28	0.002494
5α-dihydrotestosterone	0.00334	2.19E+01	1.51E+00	0.05251	1.94	0.31	0.003342
11β-hydroxyandrosterone	0.00077	3.50E+01	9.97E-01	0.00531	2.07	0.22	0.000775
17α-acetoxyprogesterone	0.00099	1.71E+01	1.01E+00	0.02564	2.06	0.24	0.000985
17α-hydroxyprogsterone	0.00499	3.74E+01	1.68E+00	0.02766	2.10	0.38	0.004995
17α-testosterone	0.00204	3.15E+01	1.28E+00	0.01545	2.09	0.29	0.002038
17α-trenbolone	0.00032	3.62E+01	8.03E-01	0.00222	1.97	0.17	0.000321
17β-testosterone	0.00727	1.29E+02	1.76E+00	0.00432	1.82	0.36	0.007266
17β-trenbolone	0.00376	3.41E+01	1.43E+00	0.01921	2.14	0.35	0.003760
19-norethindrone	0.00099	7.58E+01	1.12E+00	0.00192	2.17	0.25	0.000991
19-nortestosterone	0.00118	1.82E+02	1.20E+00	0.00060	1.39	0.17	0.001180
α-zearalenol	0.00401	9.06E+00	1.48E+00	0.22786	1.90	0.32	0.004013
α-zeranol	0.00698	3.69E+02	1.77E+00	0.00103	1.74	0.34	0.006981

		Freundlich coefficient			Freundlich exponent (n)		
Compound	**Model p-value**	**K_F**	**SD**	**p-value**	**n**	**SD**	**p-value**
β -zearalenol*							
β-zeranol	0.00027	1.01E+01	6.13E-01	0.01955	2.85	0.24	0.000275
androstenedione	0.00168	7.19E+01	1.19E+00	0.00264	2.51	0.33	0.001681
androsterone	0.06724	1.42E+02	4.12E+00	0.06079	1.59	0.64	0.067240
caproxyprogesterone	0.00083	4.36E+02	1.00E+00	0.00009	1.53	0.17	0.000828
chlorotestosterone acetate	0.00698	1.24E+03	1.24E+00	0.00006	1.26	0.25	0.006978
cortisol	0.01711	7.47E+02	3.05E+00	0.00336	0.90	0.23	0.017109
Cortisone*							
dexamethasone	0.00130	4.09E+02	1.55E+00	0.00053	1.21	0.15	0.001305
diethylstilbestrol*							
epi-androsterone	0.00048	1.29E+00	6.90E-02	0.71570	2.62	0.16	0.000477
estrone	0.00201	3.03E+01	1.45E+00	0.02531	1.84	0.26	0.002005
ethynyl testosterone	0.01314	3.19E+02	1.95E+00	0.00178	1.48	0.35	0.013144
flugestone acetate*							
fluoxymesterone	0.00950	6.82E+01	2.27E+00	0.02715	1.65	0.35	0.009504
gestodene	0.01586	1.08E+03	1.88E+00	0.00035	1.02	0.25	0.015862
medroxyprogesterone	0.00075	1.02E+02	1.01E+00	0.00077	1.73	0.19	0.000750
Medroxyprogesterone acetate							
megestrol	0.00873	1.19E+02	1.96E+00	0.00719	1.97	0.41	0.008727
megestrol acetate	0.00056	9.68E+01	9.47E-01	0.00066	1.94	0.19	0.000563
mestanolone	0.05399	1.44E+03	2.52E+00	0.00080	0.77	0.28	0.053992
methylboldenone	0.00262	3.41E+01	1.59E+00	0.02725	1.85	0.28	0.002619
methylprogesterone	0.00106	3.32E+02	8.57E-01	0.00007	2.25	0.26	0.001061
methyltestosterone	0.00044	1.45E+02	9.37E-01	0.00033	1.85	0.17	0.000443
norethandrolone	0.00715	6.08E+01	1.87E+00	0.01734	2.35	0.46	0.007154
norgestrel	0.00231	2.60E+02	1.24E+00	0.00041	1.55	0.23	0.002314
prednisolone	0.03379	6.36E+02	3.87E+00	0.00945	0.99	0.31	0.033791
progesterone	0.00971	2.02E+02	1.92E+00	0.00313	2.02	0.43	0.009709
stanozolol	0.00391	1.40E+03	1.10E+00	0.00003	1.33	0.22	0.003909
testosterone acetate	0.00487	6.96E+01	1.58E+00	0.00772	2.43	0.43	0.004869
testosterone benzoate	0.00887	4.81E+03	1.58E+00	0.00004	0.80	0.17	0.008870
testosterone propionate	0.00131	6.54E+02	1.02E+00	0.00006	1.67	0.21	0.001312

Table C4. The statistical evaluation of the response surface modelling (RSM) encompassing the significance of the interaction terms. The Asterix(*) corresponds to compounds that could not be calculated, because concentrations were detected below detection limits, resulting in in lack-of data points for constructing a reliable Box-Behnken design. T, pH and S corresponds to temperature, acidity and salinity, respectively.

	RSM			p-value of the coefficients								
compound	**P-value**	**RMSE**	**R^2**	**T**	**T*T**	**T*S**	**pH**	**pH*T**	**pH*S**	**pH*pH**	**S**	**S*S**
(alkyl)phenols												
2,5-dichloro phenol	0.6890	1.06	0.69	0.7922	0.9135	0.5188	0.8729	0.5496	0.9791	0.7418	0.1356	0.4007
bisphenol A	0.8221	1.95	0.59	0.7831	0.6917	0.9379	0.7120	0.9417	0.6137	0.2716	0.2797	0.4045
isopropyl phenol	0.7347	1.05	0.66	0.5503	0.9010	0.9510	0.9086	0.6752	0.6661	0.8133	0.1673	0.4186
trichlorophenol	0.7994	2.12	0.61	0.8302	0.3819	0.9532	0.5371	0.9983	0.6407	0.3358	0.2282	0.5676
personal care products												
DEET	0.0162	0.10	0.98	0.0028		0.1844	0.0810	0.2194	0.9939		0.0679	
ethylparaben	0.0990	0.24	0.98	0.3214	0.0152	0.5280	0.7884	0.5375	0.5406	0.0388	0.6130	0.0262
methylparaben*												
piperonylbutoxide*												
propylparaben*												
pesticides												
acetamiprid	0.0012	0.06	1.00	0.0194	0.0001	0.0797	0.2507	0.0794	0.6163	0.0003	0.1074	0.0003
alachlor*												
atrazine	0.0012	0.06	1.00	0.3130	0.0001	0.0423	0.4011	0.0469	0.5031	0.0003	0.0455	0.0003
chlorfenvinphos	0.0505	0.04	1.00	0.1061	0.0169	0.0923	0.0751	0.0731	0.4364	0.0247	0.1081	0.0246
chloridazon	0.0013	0.06	1.00	0.0070	0.0001	0.1943	0.2499	0.1150	0.9713	0.0004	0.1179	0.0003
clothianidin	0.0018	0.07	1.00	0.0045	0.0001	0.5021	0.2144	0.1119	0.7316	0.0005	0.4934	0.0004
dichlorophenoxyacetic acid	0.0319	0.08	0.97	0.0045		0.8043	0.1055	0.3132	0.1044		0.0188	
dimethoate	0.0020	0.07	1.00	0.0050	0.0002	0.5272	0.3612	0.0954	0.5826	0.0005	0.0715	0.0004
dinoseb	0.2664	0.18	0.93	0.1844		0.5711	0.4027	0.6463	0.5532		0.0747	
diuron	0.0013	0.07	1.00	0.8411	0.0001	0.0314	0.3177	0.0431	0.2879	0.0004	0.0472	0.0005
flufenacet	0.0046	0.08	0.99	0.0678	0.0004	0.1340	0.1624	0.1102	0.9822	0.0012	0.0608	0.0009
imidacloprid	0.0027	0.08	1.00	0.0316	0.0002	0.2402	0.2895	0.1415	0.8903	0.0007	0.6423	0.0007
irgarol	0.0003	0.04	1.00	0.0200	0.0000	0.0258	0.0394	0.0064	0.3510	0.0001	0.3396	0.0001
isoproturon	0.0007	0.06	1.00	0.0572	0.0001	0.0249	0.3453	0.0263	0.2522	0.0002	0.0473	0.0002
linuron	0.0014	0.07	1.00	0.3978	0.0001	0.0206	0.0598	0.0407	0.2412	0.0004	0.0354	0.0004
mecoprop	0.0058	0.09	1.00	0.0228	0.0006	0.1355	0.1233	0.6576	0.2035	0.0011	0.0510	0.0008
methiocarb	0.0202	0.10	1.00	0.2957	0.0026	0.1314	0.1102	0.1274	0.8354	0.0068	0.0861	0.0084
metolachlor	0.0053	0.10	0.99	0.4739	0.0004	0.3664	0.3127	0.1693	0.9713	0.0012	0.1174	0.0009

	RSM			p-value of the coefficients								
compound	**P-value**	**RMSE**	**R^2**	**T**	**T*T**	**T*S**	**pH**	**pH*T**	**pH*S**	**pH*pH**	**S**	**S*S**
pirimicarb	0.0074	0.11	0.99	0.9004	0.0005	0.4868	0.8422	0.1982	0.7497	0.0019	0.2499	0.0013
quinoxyfen*												
simazine	0.0008	0.06	1.00	0.0741	0.0001	0.0308	0.8042	0.0380	0.4868	0.0002	0.0321	0.0003
terbuthylazine	0.0031	0.08	0.99	0.6773	0.0002	0.1031	0.2381	0.1295	0.6491	0.0009	0.0510	0.0007
terbutryn	0.0005	0.05	1.00	0.0518	0.0000	0.0746	0.0601	0.0102	0.3827	0.0002	0.5159	0.0001
thiacloprid	0.0009	0.06	1.00	0.3852	0.0001	0.0374	0.8350	0.0437	0.1983	0.0003	0.1123	0.0003
thiamethoxam	0.0017	0.07	1.00	0.0053	0.0001	0.3002	0.4409	0.1143	0.9790	0.0004	0.3346	0.0004
pharmaceuticals												
alprazolam	0.0008	0.05	1.00	0.0041	0.0001	0.0735	0.4664	0.0556	0.2668	0.0002	0.9907	0.0002
amitriptyline	0.0204	0.20	0.98	0.3071	0.0024	0.3981	0.1854	0.1865	0.6382	0.0201	0.0208	0.0030
azithromycin	0.2317	0.25	0.89	0.2702	0.3145	0.3801	0.7552	0.9392	0.7446	0.5142	0.0391	0.1384
bezafibrate	0.0161	0.14	0.98	0.2370	0.0017	0.2502	0.5099	0.2994	0.1757	0.0029	0.0203	0.0042
bisoprolol	0.0088	0.12	0.99	0.0654	0.0007	0.3027	0.4209	0.1968	0.3506	0.0031	0.0811	0.0013
carbamazepine	0.0012	0.07	1.00	0.2916	0.0001	0.0371	0.7203	0.0521	0.3313	0.0004	0.0991	0.0004
chloramphenicol	0.0017	0.06	1.00	0.6936	0.0001	0.1116	0.4935	0.0675	0.7314	0.0004	0.3269	0.0004
clofibric acid	0.2473	0.14	0.87	0.0519		0.5074	0.0931	0.9907	0.9686		0.6266	
clarithromycin	0.0250	0.19	0.98	0.1110	0.0033	0.3546	0.3313	0.4192	0.4199	0.0116	0.0542	0.0027
diazepam	0.0009	0.05	1.00	0.0116	0.0001	0.0377	0.1786	0.0302	0.3491	0.0003	0.2013	0.0002
diclofenac	0.0319	0.18	0.97	0.9838	0.0051	0.0492	0.5066	0.3981	0.3200	0.0065	0.0265	0.0065
efavirenz*												
enrofloxacin	0.1138	0.22	0.93	0.4028		0.5776	0.5923	0.5049	0.9655		0.0154	
flumequine	0.1592	0.31	0.91	0.4017		0.2686	0.1438	0.6516	0.1720		0.0342	
fluoxetine	0.1462	0.19	0.96	0.7265		0.7003	0.2252	0.1413	0.3933		0.0445	
ifosfamide	0.0010	0.06	1.00	0.0384	0.0001	0.1435	0.3318	0.0929	0.9607	0.0003	0.1780	0.0002
indomethacin	0.3453	0.31	0.98	0.8131	0.1464	0.2936	0.8889	0.6159	0.8646	0.1589	0.3041	0.1635
ketoprofen	0.0050	0.08	0.99	0.6530	0.0006	0.0427	0.4376	0.2595	0.1511	0.0007	0.0105	0.0009
metoprolol	0.0035	0.09	0.99	0.2610	0.0002	0.3155	0.2671	0.0985	0.5444	0.0012	0.1223	0.0006
moxifloxacin	0.1555	0.26	0.91	0.6456		0.5014	0.9210	0.5850	0.9019		0.0226	
nalidixic acid	0.0959	0.34	0.94	0.3627	0.0298	0.3145	0.1472	0.6229	0.1901	0.0697	0.0474	0.0156
nevirapine	0.0014	0.06	1.00	0.0824	0.0001	0.0568	0.7739	0.0536	0.4201	0.0003	0.2030	0.0003
oseltamivir ethylester	0.0207	0.15	0.98	0.1210	0.0017	0.3551	0.6190	0.3288	0.6456	0.0084	0.1774	0.0030
paroxetine	0.0923	0.24	0.98	0.8732	0.0193	0.8722	0.2453	0.2271	0.7950	0.0635	0.0830	0.0182
propranolol	0.0071	0.12	0.99	0.3317	0.0005	0.3132	0.2043	0.1229	0.2543	0.0045	0.0324	0.0013
rimantadine	0.0139	0.13	0.98	0.2130	0.0009	0.6789	0.2345	0.2445	0.6969	0.0081	0.2106	0.0032

	RSM			p-value of the coefficients								
compound	**P-value**	**RMSE**	**R^2**	**T**	**T*T**	**T*S**	**pH**	**pH*T**	**pH*S**	**pH*pH**	**S**	**S*S**
sarafloxacin	0.1580	0.31	0.92	0.8921	0.0344	0.5117	0.7399	0.6303	0.7466	0.1248	0.0761	0.0201
sulfadoxin	0.0093	0.13	0.99	0.6681	0.6607	0.6583	0.6355	0.6877	0.6234	0.6171	0.4081	0.6562
sulfamethazine	0.0030	0.08	0.99	0.0906	0.0003	0.0805	0.0146	0.2768	0.0988	0.0009	0.2479	0.0005
sulfamethoxazole	0.0302	0.24	0.97	0.0596	0.0116	0.2270	0.0161	0.3407	0.1182	0.0217	0.0348	0.0056
tetracycline	0.7754	0.47	0.6	0.8360		0.6079	0.8597	0.9773	0.8187		0.2998	
trimethoprim	0.0017	0.07	1.00	0.8736	0.0001	0.3330	0.0199	0.0378	0.4921	0.0007	0.4203	0.0005
phthalates												
benzylbutyl phthalate	0.7516	1.34	0.65	0.7053	0.7654	0.9100	0.5129	0.8668	0.6109	0.2671	0.2139	0.6525
diamyl phthalate	0.8666	1.86	0.55	0.5410	0.7451	0.6789	0.9123	0.5351	0.8302	0.6860	0.3419	0.5524
dibutyl phthalate	0.7365	1.53	0.66	0.9017	0.5871	0.8994	0.5101	0.9423	0.5234	0.2461	0.2177	0.4241
dicyclohexyl phthalate	0.7323	1.50	0.66	0.8186	0.6493	0.9241	0.5638	0.8998	0.4968	0.2373	0.2129	0.6748
diethyl phthalate	0.4431	0.46	0.8	0.4960	0.7128	0.6295	0.3735	0.6295	0.9839	0.2004	0.1236	0.5842
dihexyl phthalate	0.9075	1.33	0.51	0.5826	0.9635	0.8683	0.7292	0.7859	0.5768	0.4794	0.3698	0.9177
diethylheyxl phthalate	0.4812	1.08	0.79	0.8404	0.3154	0.9796	0.1259	0.9422	0.4612	0.1480	0.2719	0.5926
dimethyl phthalate	0.6905	0.81	0.68	0.5379	0.7674	0.5823	0.2607	0.7809	0.9909	0.3065	0.4188	0.8163
dinonyl phthalate*												
monobenzyl phthalate	0.7771	3.04	0.63	0.9957	0.6739	0.6210	0.07541	0.4256	0.8893	0.6692	0.3413	0.2095
monocyclohexyl phthalate	0.1830	1.01	0.91	0.9432	0.4440	0.6595	0.0372	0.2779	0.1715	0.0625	0.3109	0.1512
monoethyl phthalate	0.6745	0.93	0.69	0.9113	0.4911	0.9489	0.9058	0.5758	0.6941	0.1346	0.3885	0.9866
monohexyl phthalate	0.7814	1.57	0.63	0.7711	0.9292	0.8489	0.3419	0.8375	0.6708	0.2573	0.3900	0.8506
monomethyl phthalate	0.6318	0.83	0.72	0.5888	0.5331	0.9201	0.3582	0.6807	0.9556	0.2203	0.1776	0.8846
mono-n-pentyl phthalate	0.1518	0.66	0.92	0.6305	0.3827	0.7284	0.0326	0.3851	0.0578	0.0594	0.7936	0.2162
steroidal EDCs												
1,4-androstadienedione	0.0526	0.29	0.96	0.0118	0.0303	0.9112	0.0571	0.0421	0.7402	0.0207	0.7051	0.0645
11-keteotiocholanolone	0.0165	0.14	0.98	0.0072	0.0030	0.7192	0.5421	0.1644	0.8949	0.0030	0.1256	0.0036
11-ketotestosterone	0.0043	0.09	0.99	0.0009	0.0013	0.9216	0.2105	0.0372	0.1283	0.0012	0.0619	0.0014
5α-dihydrotestosterone	0.0002	0.03	0.99	0.0001	0.0001	0.0456	0.0045	0.0018	0.0367	0.0001	0.0078	0.0001
11β-hydroxyandrosterone	0.0830	0.36	0.95	0.0268	0.0239	1.0000	0.0819	0.2101	0.4324	0.0292	0.9148	0.0239
17α-acetoxyprogesterone	0.0054	0.12	0.99	0.0014	0.0020	0.4518	0.0313	0.0422	0.2683	0.0015	0.0454	0.0013
17β-hydroxyprogesterone	0.0203	0.18	0.98	0.0052	0.0056	0.7292	0.1207	0.0992	0.3469	0.0050	0.1425	0.0073
17α-testosterone	0.2753	0.62	0.87	0.0574	0.1091	0.9294	0.2194	0.9882	0.9823	0.1061	0.9376	0.0972
17α-trenbolone	0.3735	0.55	0.83	0.9859	0.1035	0.0981	0.7521	0.1852	0.7970	0.5462	0.9953	0.2224
17β-testosterone	0.0294	0.20	0.97	0.0081	0.0073	0.8740	0.1365	0.1741	0.1741	0.0092	0.2188	0.0082
17β-trenbolone	0.0343	0.18	0.97	0.0073	0.0109	0.9004	0.2654	0.2669	0.4112	0.0090	0.6513	0.0093

	RSM			p-value of the coefficients								
compound	**P-value**	**RMSE**	**R^2**	**T**	**T*T**	**T*S**	**pH**	**pH*T**	**pH*S**	**pH*pH**	**S**	**S*S**
19-norethindron	0.0006	0.05	1.00	0.0002	0.0003	0.4969	0.0018	0.0055	0.0170	0.0002	0.0113	0.0002
19-nortestosterone	0.6009	0.57	0.73	0.6444	0.1497	0.7122	0.5700	0.6891	0.7717	0.1367	0.7083	0.1131
α-zearalenol	0.0336	0.22	0.97	0.0097	0.0087	0.7389	0.2723	0.1158	0.1134	0.0112	0.0646	0.0171
α-zeranol	0.1397	0.12	0.92	0.0269	0.7399	0.1233	0.0470	0.4421	0.4611	0.7813	0.4056	0.3904
β-zearalenol												
β-zeranol	0.2094	0.22	0.90	0.2003	0.0416	0.7716	0.7047	0.8351	0.8351	0.0350	0.6633	0.0278
androstenedione	0.0060	0.10	0.99	0.0029	0.0011	0.7417	0.0862	0.1130	0.3777	0.0010	0.1050	0.0012
androsterone*												
caproxyprogesterone	0.0248	0.28	0.98	0.0195	0.0095	0.3870	0.0585	0.1440	0.0907	0.0060	0.1354	0.0037
chlorotestosteron acetate	0.2512	0.51	0.88	0.0969	0.1412	0.2603	0.4853	0.5467	0.9008	0.0582	0.9447	0.0522
cortisol	0.0061	0.13	0.99	0.0033	0.0018	0.7089	0.0111	0.0372	0.3583	0.0011	0.0743	0.0014
cortisone*												
dexamethasone*												
diesthylstilbestrol*												
epi-androsterone*												
estrone	0.0382	0.28	0.97	0.0133	0.0415	0.9474	0.1057	0.1485	0.0758	0.0087	0.1278	0.0115
ethynyl testosterone	0.0053	0.08	0.99	0.0009	0.0019	0.8186	0.0483	0.0829	0.3907	0.0023	0.1603	0.0014
flugestone acetate	0.3422	0.39	0.84	0.1121	0.8065	0.3949	0.1075	0.7600	0.3689	0.2079	0.5146	0.9393
fluoxymesterone	0.0043	0.10	0.99	0.0009	0.0017	0.8929	0.0169	0.0405	0.2057	0.0011	0.1007	0.0014
gestone	0.1631	0.62	0.91	0.0350	0.0863	0.9525	0.2734	0.8126	0.6608	0.0639	0.3612	0.0389
medroxyprogesterone	0.0126	0.15	0.99	0.0024	0.0045	0.3905	0.1108	0.2147	0.0366	0.0046	0.1294	0.0053
medroxyprogesterone acetate	0.0364	0.27	0.97	0.0069	0.0096	0.6155	0.1340	0.1217	0.7096	0.0253	0.1584	0.0167
megestrol	0.0765	0.36	0.95	0.0179	0.0349	0.5810	0.1298	0.3097	0.4141	0.0205	0.5903	0.0248
megestrol acetate	0.0057	0.14	0.99	0.0015	0.0015	0.9197	0.0128	0.0124	0.8148	0.0022	0.0748	0.0029
mestanolone	0.0288	0.32	0.98	0.0059	0.0123	0.6512	0.8550	0.4835	0.8519	0.0075	0.1128	0.0071
methylboldone	0.0128	0.14	0.99	0.0020	0.0040	0.4800	0.0725	0.0837	0.7261	0.0057	0.3561	0.0080
methylprogesterone	0.2234	0.41	0.89	0.0602	0.0678	0.3777	0.7364	0.6153	0.9198	0.1950	0.1578	0.0738
norethandrolone	0.0406	0.18	0.97	0.0074	0.0136	0.4858	0.3512	0.1774	0.7372	0.0197	0.0906	0.0165
norgestrel	0.0045	0.08	0.99	0.0009	0.0014	0.8243	0.0337	0.0565	0.2275	0.0018	0.1171	0.0014
prednisolone	0.0317	0.26	0.97	0.0122	0.0099	0.2950	0.0636	0.1090	0.6846	0.0089	0.1495	0.0073
prednisone*												
progesterone	0.0044	0.13	0.99	0.0010	0.0013	0.3307	0.0531	0.0155	0.4262	0.0015	0.1212	0.0013
stanozolol												

	RSM			p-value of the coefficients								
compound	**P-value**	**RMSE**	**R^2**	**T**	**T*T**	**T*S**	**pH**	**pH*T**	**pH*S**	**pH*pH**	**S**	**S*S**
testosterone phenylpropionate*												
testosterone acetate	0.2234	0.41	0.89	0.0602	0.0678	0.3777	0.7364	0.6153	0.9198	0.1950	0.1578	0.0738
testosterone benzoate	0.1323	0.35	0.93	0.8145	0.0154	0.3460	0.3980	0.6745	0.3204	0.0280	0.3456	0.0232
testosterone propionate	0.0495	0.30	0.96	0.0128	0.0116	0.7187	0.3757	0.7187	0.8914	0.0129	0.1226	0.0156
trenbolone acetate*												

Table C5. The coefficients of the obtained response surface models. The Asterix(*) corresponds to compounds that could not be calculated, because concentrations were detected below detection limits, resulting in in lack-of data points for constructing a reliable Box-Behnken design. T, pH and S corresponds to temperature, acidity and salinity, respectively.

compound	Intercept	T	T*T	T*S	pH	pH*T	pH*S	pH*pH	S	S*S
alkylphenols										
2,5-dichloro phenol	4.60E+00	-1.08E-01	-8.25E-02	-3.85E-01	-6.50E-02	3.55E-01	-1.50E-02	2.53E-01	-7.58E-01	6.83E-01
bisphenol A	8.26E+00	2.08E-01	-5.64E-01	-8.25E-02	-2.80E-01	7.75E-02	5.48E-01	-1.73E+00	-9.08E-01	-1.25E+00
isopropyl phenol	4.88E+00	2.49E-01	-9.38E-02	-3.50E-02	-4.63E-02	2.43E-01	2.50E-01	-1.79E-01	-6.73E-01	6.49E-01
trichlorophenol	6.61E+00	-8.00E-02	-5.25E-01	-7.50E-03	-3.39E-01	6.75E-02	3.60E-01	-1.26E+00	-8.56E-01	-4.38E-01
personal care products										
DEET	4.86E+00	-3.30E-01	3.35E-01	8.81E-02	9.40E-02	7.94E-02	4.25E-04	-1.96E-01	1.02E-01	0.00E+00
ethylparaben	3.51E+00	-1.33E-01	1.33E+00	-1.26E-01	-2.54E-02	8.67E-02	-8.60E-02	8.19E-01	-6.04E-02	1.01E+00
methylparaben*										
piperonylbutoxide*										
propylparaben*										
pesticides										
acetamiprid	3.22E+00	-9.83E-02	1.24E+00	-7.90E-02	-3.04E-02	7.91E-02	-1.69E-02	7.61E-01	4.87E-02	7.71E-01
alachlor*										
atrazine	3.31E+00	2.75E-02	1.30E+00	-1.09E-01	2.22E-02	1.05E-01	-2.44E-02	8.01E-01	7.51E-02	7.91E-01
chlorfenvinphos	3.71E+00	1.36E-01	1.16E+00	-1.95E-01	1.43E-01	2.47E-01	2.28E-02	6.91E-01	9.92E-02	6.95E-01
chloridazon	3.10E+00	-1.43E-01	1.17E+00	-5.09E-02	-3.07E-02	6.72E-02	-1.19E-03	7.42E-01	4.70E-02	7.75E-01
clothianidin	2.92E+00	-1.78E-01	1.08E+00	-2.48E-02	-3.63E-02	7.29E-02	1.23E-02	7.27E-01	1.80E-02	7.74E-01
dichlorophenoxyacetic acid	4.16E+00	-2.27E-01	9.92E-02	-1.13E-02	-6.75E-02	5.03E-02	9.60E-02	-1.75E-02	1.37E-01	0.00E+00
dimethoate	2.84E+00	-1.88E-01	1.15E+00	-2.55E-02	-2.72E-02	8.60E-02	2.20E-02	7.65E-01	6.92E-02	8.14E-01
dinoseb	4.80E+00	-1.54E-01	3.67E-01	-8.49E-02	6.66E-02	4.78E-02	6.31E-02	9.80E-02	2.67E-01	0.00E+00
diuron	3.71E+00	5.07E-03	1.30E+00	-1.26E-01	2.77E-02	1.11E-01	-4.23E-02	7.48E-01	7.55E-02	7.27E-01
flufenacet	3.41E+00	8.40E-02	1.04E+00	-8.66E-02	5.53E-02	9.54E-02	-1.03E-03	6.91E-01	8.80E-02	7.66E-01
imidacloprid	3.12E+00	-1.03E-01	1.18E+00	-5.59E-02	-3.48E-02	7.60E-02	-5.74E-03	7.25E-01	1.39E-02	7.37E-01
irgarol	3.61E+00	5.90E-02	1.23E+00	-7.58E-02	4.55E-02	1.26E-01	-2.02E-02	6.63E-01	1.47E-02	8.04E-01
linuron	3.69E+00	-2.30E-02	1.27E+00	-1.49E-01	6.91E-02	1.14E-01	-4.82E-02	7.65E-01	8.56E-02	7.69E-01
mecoprop	2.75E+00	-1.37E-01	9.08E-01	-9.09E-02	-6.74E-02	2.20E-02	7.26E-02	7.46E-01	1.00E-01	8.12E-01
methiocarb	3.57E+00	6.06E-02	1.38E+00	-1.24E-01	1.19E-01	1.78E-01	1.18E-02	8.49E-01	1.12E-01	7.68E-01
metolachlor	3.20E+00	2.88E-02	1.19E+00	-5.30E-02	4.27E-02	9.00E-02	1.95E-03	7.97E-01	7.69E-02	8.78E-01
pirimicarb	3.13E+00	-5.42E-03	1.20E+00	-4.45E-02	8.63E-03	9.27E-02	-1.97E-02	7.69E-01	5.66E-02	8.89E-01
quinoxyfen*										

compound	Intercept	T	T*T	T*S	pH	pH*T	pH*S	pH*pH	S	S*S
simazine	3.30E+00	-5.27E-02	1.26E+00	-1.07E-01	-5.29E-03	9.82E-02	-2.19E-02	7.64E-01	7.43E-02	7.53E-01
terbuthylazine	3.54E+00	1.34E-02	1.20E+00	-9.60E-02	4.30E-02	8.59E-02	-2.09E-02	7.30E-01	9.24E-02	7.90E-01
terbutryn	3.60E+00	5.00E-02	1.24E+00	-6.06E-02	4.70E-02	1.31E-01	-2.30E-02	6.67E-01	1.17E-02	8.14E-01
thiacloprid	3.50E+00	-2.11E-02	1.34E+00	-1.05E-01	-4.73E-03	9.89E-02	-4.85E-02	7.84E-01	4.64E-02	7.57E-01
thiamethoxam	2.71E+00	-1.71E-01	1.14E+00	-4.14E-02	-2.07E-02	7.31E-02	-9.48E-04	7.72E-01	2.69E-02	8.05E-01
pharmaceuticals										
alprazolam	3.10E+00	1.51E-01	1.18E+00	-7.25E-02	1.58E-02	8.16E-02	-3.65E-02	7.52E-01	-2.40E-04	8.42E-01
amitriptyline	3.28E+00	8.74E-02	1.28E+00	-9.90E-02	1.22E-01	1.72E-01	-5.25E-02	6.04E-01	-3.19E-01	1.19E+00
azithromycin	2.83E+00	-1.19E-01	-1.99E-01	1.28E-01	-3.01E-02	-1.03E-02	4.46E-02	1.22E-01	3.10E-01	-3.31E-01
bezafibrate	3.04E+00	7.13E-02	9.86E-01	-9.73E-02	3.61E-02	8.56E-02	1.21E-01	8.17E-01	2.18E-01	7.19E-01
bisoprolol	2.99E+00	1.22E-01	1.14E+00	-7.51E-02	3.98E-02	1.00E-01	-6.67E-02	7.04E-01	-1.11E-01	9.56E-01
carbamazepine	3.42E+00	2.95E-02	1.33E+00	-1.17E-01	9.09E-03	1.02E-01	-3.78E-02	7.93E-01	5.46E-02	7.67E-01
chloramphenicol	3.16E+00	9.72E-03	1.13E+00	-7.08E-02	-1.74E-02	8.90E-02	-1.19E-02	7.35E-01	2.62E-02	7.56E-01
clofibric acid	4.26E+00	-1.52E-01	-3.01E-02	-5.14E-02	-1.18E-01	8.70E-04	-2.93E-03	-1.60E-01	2.61E-02	0.00E+00
clarithromycin	2.63E+00	1.49E-01	1.07E+00	-1.03E-01	7.68E-02	8.77E-02	-8.75E-02	6.88E-01	-2.04E-01	1.14E+00
diazepam	3.45E+00	1.04E-01	1.14E+00	-9.43E-02	3.27E-02	1.03E-01	-2.93E-02	7.09E-01	3.05E-02	7.37E-01
diclofenac	3.43E+00	1.39E-03	8.69E-01	-2.85E-01	4.73E-02	8.74E-02	1.06E-01	8.01E-01	2.57E-01	8.00E-01
efavirenz*										
enrofloxacin	4.95E+00	7.59E-02	-2.37E-01	-6.88E-02	4.67E-02	8.34E-02	5.18E-03	-5.11E-01	-3.90E-01	0.00E+00
flumequine	4.87E+00	-1.08E-01	-2.32E-01	-2.12E-01	-2.18E-01	-7.82E-02	-2.80E-01	-5.00E-01	-4.10E-01	0.00E+00
fluoxetine	5.58E+00	3.22E-02	-1.46E-02	5.81E-02	1.13E-01	2.19E-01	9.99E-02	-5.59E-01	-3.67E-01	0.00E+00
ifosfamide	2.73E+00	-7.02E-02	1.18E+00	-5.52E-02	-2.29E-02	6.83E-02	-1.50E-03	7.65E-01	3.47E-02	7.93E-01
indomethacin	3.24E+00	3.29E-02	9.87E-01	-3.10E-01	2.72E-02	1.06E-01	5.76E-02	9.06E-01	2.98E-01	8.80E-01
isoproturon	3.41E+00	5.89E-02	1.33E+00	-1.16E-01	2.18E-02	1.13E-01	-3.91E-02	8.03E-01	6.36E-02	7.75E-01
ketoprofen	3.26E+00	1.46E-02	8.59E-01	-1.41E-01	-2.62E-02	5.75E-02	7.95E-02	7.88E-01	1.68E-01	7.36E-01
metoprolol	3.05E+00	4.22E-02	1.20E+00	-5.19E-02	4.15E-02	1.02E-01	-2.94E-02	6.87E-01	-6.52E-02	8.65E-01
moxifloxacin	4.88E+00	4.73E-02	-1.99E-01	-1.00E-01	9.99E-03	8.00E-02	-1.76E-02	-5.61E-01	-4.03E-01	0.00E+00
nalidixic acid	2.42E+00	-1.28E-01	8.76E-01	-2.04E-01	-2.33E-01	-9.26E-02	-2.86E-01	6.21E-01	-3.90E-01	1.12E+00
nevirapine	3.17E+00	5.80E-02	1.21E+00	-9.63E-02	7.09E-03	9.86E-02	-2.97E-02	7.81E-01	3.66E-02	7.89E-01
oseltamivir ethylester	2.84E+00	1.17E-01	1.10E+00	-8.43E-02	3.02E-02	8.99E-02	3.93E-02	6.34E-01	-9.59E-02	9.06E-01
paroxetine	3.35E+00	-1.84E-02	1.18E+00	3.03E-02	1.35E-01	2.03E-01	3.48E-02	6.28E-01	-3.31E-01	1.22E+00
propranolol	3.41E+00	5.00E-02	1.29E+00	-7.40E-02	7.00E-02	1.30E-01	-8.61E-02	6.26E-01	-1.64E-01	9.55E-01
rimantadine	3.03E+00	-7.38E-02	1.16E+00	-3.03E-02	6.95E-02	9.56E-02	2.84E-02	5.52E-01	-7.43E-02	7.60E-01
sarafloxacin	2.92E+00	-1.61E-02	7.54E-01	-1.15E-01	3.97E-02	8.24E-02	-5.46E-02	4.31E-01	-2.91E-01	9.26E-01
sulfadoxin	2.56E+00	-6.72E-02	9.95E-01	-1.36E-01	-2.67E-01	-6.52E-02	-1.52E-01	7.28E-01	-1.47E-01	1.01E+00

compound	Intercept	T	T*T	T*S	pH	pH*T	pH*S	pH*pH	S	S*S
sulfamethazine	2.87E+00	-6.82E-02	1.07E+00	-1.02E-01	-1.41E-01	5.19E-02	-9.26E-02	6.96E-01	-3.96E-02	8.58E-01
sulfamethoxazole	2.34E+00	-2.48E-01	8.69E-01	-1.79E-01	-4.12E-01	-1.34E-01	-2.57E-01	6.90E-01	-3.08E-01	1.12E+00
tetracycline	4.44E+00	3.73E-02	-3.43E-01	-1.34E-01	-3.18E-02	-7.23E-03	-5.85E-02	-5.01E-01	-2.07E-01	0.00E+00
trimethoprim	3.21E+00	-4.22E-03	1.25E+00	-3.97E-02	1.11E-01	1.23E-01	2.69E-02	6.71E-01	-2.27E-02	7.65E-01
phthalates										
benzyl butyl phthalate	6.78E+00	-1.98E-01	-2.90E-01	-8.25E-02	-3.51E-01	1.23E-01	3.80E-01	-1.21E+00	-7.46E-01	-4.43E-01
diamyl phthalate	4.94E+00	-4.53E-01	4.39E-01	4.25E-01	-7.88E-02	6.50E-01	2.18E-01	-5.49E-01	-7.41E-01	8.21E-01
dibenzyl phthalate	4.60E+00	-1.01E-01	-1.13E-02	3.30E-01	-7.88E-02	1.43E-01	1.50E-02	1.24E-01	-6.25E-02	-1.09E-01
dibutyl phthalate	6.92E+00	-7.25E-02	-6.13E-01	-1.05E-01	-4.03E-01	6.00E-02	5.50E-01	-1.45E+00	-8.40E-01	-9.33E-01
dicyclohexyl phthalate	6.79E+00	-1.33E-01	-4.99E-01	-7.75E-02	-3.43E-01	1.03E-01	5.78E-01	-1.46E+00	-8.35E-01	-4.59E-01
diethyl phthalate	5.39E+00	-1.25E-01	1.23E-01	1.23E-01	-1.69E-01	1.23E-01	-5.00E-03	-4.95E-01	-3.44E-01	1.85E-01
diethylheyxl phthalate	6.48E+00	-8.38E-02	-8.59E-01	-1.50E-02	-8.04E-01	-4.25E-02	4.55E-01	-1.38E+00	-5.13E-01	-4.26E-01
dihexyl phthalate	5.97E+00	-2.89E-01	-4.38E-02	-1.20E-01	-1.79E-01	1.98E-01	4.15E-01	-7.09E-01	-4.95E-01	9.88E-02
dimethyl phthalate	5.61E+00	-1.98E-01	1.73E-01	2.48E-01	-3.94E-01	-1.23E-01	-5.00E-03	-6.55E-01	-2.66E-01	1.35E-01
monobenzyl phthalate	3.98E+00	-3.69E-01	9.49E-01	-2.30E-01	3.25E+00	-1.21E+00	-1.40E+00	8.35E-01	-6.25E-03	9.34E-01
monocyclohexyl phthalate	6.88E+00	2.75E-02	-5.85E-01	2.45E-01	-1.28E+00	-6.65E-01	9.00E-01	-1.93E+00	-4.33E-01	-1.28E+00
monoethyl phthalate	6.25E+00	-4.00E-02	-4.84E-01	3.25E-02	-4.25E-02	-2.93E-01	2.03E-01	-1.26E+00	-3.33E-01	1.13E-02
monohexyl phthalate	6.69E+00	-1.76E-01	-1.00E-01	-1.63E-01	-6.24E-01	1.75E-01	-3.68E-01	-1.45E+00	-5.55E-01	-2.13E-01
monomethyl phthalate	6.27E+00	-1.76E-01	-3.84E-01	4.50E-02	-3.16E-01	1.88E-01	-2.50E-02	-8.44E-01	-5.13E-01	-8.63E-02
mono-n-pentyl phthalate	6.37E+00	-1.24E-01	-4.43E-01	1.25E-01	-8.75E-01	-3.33E-01	9.83E-01	-1.29E+00	6.63E-02	-6.78E-01
steroidal EDCs										
1,4-androstadienedione	5.06E+00	-5.63E-01	-7.41E-01	1.75E-02	3.08E-01	4.93E-01	5.25E-02	-8.56E-01	-4.25E-02	-5.46E-01
11-keteotiocholanolone	5.30E+00	-3.23E-01	-8.20E-01	2.75E-02	3.38E-02	1.28E-01	1.00E-02	-8.18E-01	-1.04E-01	-7.68E-01
11-ketotestosterone	5.19E+00	-4.46E-01	-7.33E-01	5.00E-03	5.25E-02	1.68E-01	9.75E-02	-7.55E-01	-9.63E-02	-7.23E-01
5α-dihydrotestosterone*	5.35E+00	-4.24E-01	-7.89E-01	5.50E-02	9.13E-02	1.78E-01	6.00E-02	-8.04E-01	-7.50E-02	-7.36E-01
11β-hydroxyandrosterone	5.36E+00	-5.25E-01	-1.03E+00	0.00E+00	3.33E-01	2.90E-01	-1.65E-01	-9.50E-01	1.50E-02	-1.03E+00
17α-acetoxyprogesterone	5.56E+00	-4.98E-01	-8.16E-01	-5.25E-02	1.65E-01	2.08E-01	8.25E-02	-9.11E-01	-1.43E-01	-9.61E-01
17β-hydroxyprogesterone	5.40E+00	-4.79E-01	-8.73E-01	3.50E-02	1.40E-01	2.18E-01	1.03E-01	-9.10E-01	-1.29E-01	-7.98E-01
17α-testosterone	5.94E+00	-6.63E-01	-9.31E-01	3.00E-02	3.41E-01	5.00E-03	7.50E-03	-9.44E-01	1.88E-02	-9.84E-01
17α-trenbolone	4.77E+00	-3.75E-03	-8.45E-01	-6.55E-01	-6.75E-02	4.73E-01	7.75E-02	-2.48E-01	1.25E-03	-5.60E-01
17β-testosterone	3.19E+00	4.51E-01	8.76E-01	-1.75E-02	-1.45E-01	-1.80E-01	-1.80E-01	8.09E-01	1.11E-01	8.41E-01
17β-trenbolone	5.27E+00	-4.24E-01	-6.88E-01	1.25E-02	8.88E-02	1.25E-01	8.75E-02	-7.38E-01	-3.25E-02	-7.30E-01
19-norethindron	5.29E+00	-4.44E-01	-6.79E-01	-2.00E-02	1.94E-01	1.88E-01	1.25E-01	-7.69E-01	-1.03E-01	-7.76E-01
19-nortestosterone	5.37E+00	-1.03E-01	-7.23E-01	1.15E-01	1.28E-01	1.25E-01	9.00E-02	-7.58E-01	-8.25E-02	-8.33E-01
α-zearalenol	5.39E+00	-4.58E-01	-8.89E-01	-4.00E-02	1.04E-01	2.40E-01	2.43E-01	-8.11E-01	-2.21E-01	-6.96E-01

compound	Intercept	T	T*T	T*S	pH	pH*T	pH*S	pH*pH	S	S*S
α-zeranol	3.60E+00	-1.79E-01	3.00E-02	1.33E-01	1.44E-01	-5.50E-02	5.25E-02	2.50E-02	-4.25E-02	-8.25E-02
β-zearalenol*										
β-zeranol	4.90E+00	-1.28E-01	-5.00E-01	3.50E-02	3.25E-02	-2.50E-02	2.50E-02	-5.35E-01	-3.75E-02	-5.85E-01
androstenedione	5.35E+00	-3.08E-01	-8.00E-01	-1.75E-02	8.63E-02	1.08E-01	5.00E-02	-8.18E-01	-7.88E-02	-7.83E-01
caproxyprogesterone	5.65E+00	-4.58E-01	-1.11E+00	1.43E-01	2.98E-01	2.78E-01	-3.48E-01	-1.31E+00	-2.03E-01	-1.55E+00
chlorotestosteron acetate	5.09E+00	-4.36E-01	-6.79E-01	3.58E-01	1.45E-01	-1.75E-01	-3.50E-02	-1.02E+00	-1.38E-02	-1.07E+00
cortisol	5.87E+00	-4.09E-01	-9.33E-01	-2.75E-02	2.66E-01	2.40E-01	-7.25E-02	-1.11E+00	-1.28E-01	-1.02E+00
cortisone*										
dexamethasone*										
dienostrol*										
diethylstilbestrol*										
epi-androsterone										
estrone	5.39E+00	-5.20E-01	-6.34E-01	-1.00E-02	2.26E-01	2.70E-01	3.73E-01	-1.13E+00	-2.06E-01	-1.03E+00
ethynyl_testosterone	4.98E+00	-3.76E-01	-5.54E-01	1.00E-02	9.13E-02	1.03E-01	4.00E-02	-5.19E-01	-5.25E-02	-6.06E-01
flugestone acetate	4.64E+00	-3.06E-01	-6.88E-02	1.93E-01	3.13E-01	6.50E-02	-2.05E-01	-4.11E-01	1.01E-01	2.13E-02
fluoxymesterone	5.32E+00	-4.83E-01	-7.29E-01	7.50E-03	1.75E-01	1.78E-01	8.25E-02	-8.44E-01	-8.50E-02	-7.74E-01
gestone	5.76E+00	-8.03E-01	-1.03E+00	2.00E-02	2.93E-01	8.00E-02	1.50E-01	-1.18E+00	-2.35E-01	-1.44E+00
medroxyprogesterone	5.17E+00	-5.06E-01	-7.64E-01	-7.50E-02	1.19E-01	1.18E-01	2.70E-01	-7.59E-01	-1.10E-01	-7.26E-01
medroxyprogesterone acetate	5.37E+00	-6.31E-01	-1.05E+00	-7.50E-02	1.94E-01	2.88E-01	5.50E-02	-7.39E-01	-1.78E-01	-8.61E-01
megestrol	5.43E+00	-6.10E-01	-8.86E-01	-1.13E-01	2.68E-01	2.23E-01	-1.73E-01	-1.09E+00	-7.75E-02	-1.01E+00
megestrol acetate	5.36E+00	-5.49E-01	-1.02E+00	7.50E-03	2.59E-01	3.70E-01	1.75E-02	-8.90E-01	-1.30E-01	-8.13E-01
mestanolone	5.78E+00	-7.94E-01	-1.15E+00	8.00E-02	2.25E-02	1.28E-01	-3.25E-02	-1.37E+00	-2.51E-01	-1.39E+00
methylboldone	5.19E+00	-5.20E-01	-7.59E-01	5.75E-02	1.38E-01	1.83E-01	2.75E-02	-6.74E-01	-5.50E-02	-5.99E-01
methylprogesterone	4.83E+00	-4.29E-01	-7.63E-01	-2.13E-01	5.38E-02	1.15E-01	2.25E-02	-4.53E-01	-2.73E-01	-7.35E-01
methyltestosterone	5.38E+00	-5.75E-01	-9.41E-01	-5.50E-02	2.24E-01	3.15E-01	1.28E-01	-9.69E-01	-1.79E-01	-7.69E-01
norethandrolone	5.01E+00	-4.05E-01	-6.11E-01	7.00E-02	6.88E-02	1.55E-01	3.25E-02	-5.34E-01	-1.54E-01	-5.69E-01
norgestrel	5.09E+00	-3.98E-01	-6.39E-01	1.00E-02	1.09E-01	1.25E-01	6.25E-02	-5.81E-01	-6.38E-02	-6.36E-01
prednisolone	5.89E+00	-4.94E-01	-9.95E-01	-1.63E-01	2.61E-01	2.90E-01	-5.75E-02	-1.04E+00	-1.75E-01	-1.11E+00
progesterone	5.39E+00	-5.78E-01	-9.89E-01	7.25E-02	1.38E-01	3.13E-01	5.75E-02	-9.29E-01	-9.50E-02	-9.84E-01
stanozolol*										
testosterone phenylpropionate*										
testosterone acetate	4.83E+00	-4.29E-01	-7.63E-01	-2.13E-01	5.38E-02	1.15E-01	2.25E-02	-4.53E-01	-2.73E-01	-7.35E-01
testosterone benzoate	5.11E+00	-3.13E-02	-1.14E+00	1.93E-01	1.20E-01	8.00E-02	-2.05E-01	-9.14E-01	-1.36E-01	-9.81E-01
testosterone propionate	5.08E+00	-5.73E-01	-1.11E+00	6.00E-02	1.11E-01	6.00E-02	-2.25E-02	-1.07E+00	-2.29E-01	-9.99E-01

compound	Intercept	T	T*T	T*S	pH	pH*T	pH*S	pH*pH	S	S*S
trenbolone acetate										

Table C6. The calculated thermodynamic parameters, i.e. change in enthalpy ΔH (kJ/mol) , entropy ΔS (J/mol/K) and free Gibbs energy $\Delta G_{288.15K}$ (kJ/mol) (n=number of available data-points during sorption for the calculations). The Asterix(*) corresponds to compounds that could not be calculated, because concentrations were detected below detection limits, resulting in in lack-of data points for constructing a reliable Box-Behnken design. T, pH and S corresponds to temperature, acidity and salinity, respectively.

	$\Delta H_{288.15}$ (kJ/mol)			ΔS (J/mol/K)			$\Delta G_{288.15K}$ (kJ/mol)		
Compound	**Mean**	**SD**	**n**	**Mean**	**SD**	**n**	**Mean**	**SD**	**n**
alkylphenols									
2,5-dichloro phenol	-71.4	55.1	2	-2.5	23.2	2	-70.7	55.1	2
bisphenol A	-39.8		1	12.4		1	-43.4		1
isopropyl phenol	-52.2		1	5.2		1	-53.7		1
trichlorophenol	-61.7	24.4	2	-12.2	9.6	2	-58.2	24.4	2
personal care products									
DEET	-51.6	12.5	2	-17.2	5.5	2	-46.6	12.5	2
ethylparaben	-32.9	13.8	2	-8.5	5.8	2	-30.5	13.8	2
methylparaben*									
piperonylbutoxide	-13.6		1	-0.4		1	-13.5		1
propylparaben*									
pesticides									
acetamiprid	-36.9	40.2	2	12.0	16.9	2	-40.4	40.2	2
alachlor*									
atrazine	-8.2	58.5	2	24.5	24.8	2	-15.2	58.5	2
chlorfenvinphos	-13.7		1	-0.6		1	-13.5		1
chloridazon	-46.8	37.8	2	7.4	15.9	2	-48.9	37.8	2
clothianidin	-56.3	38.1	2	2.9	15.9	2	-57.1	38.1	2
dichlorophenoxyacetic acid	-34.6	7.8	2	-10.4	3.2	2	-31.6	7.8	2
dimethoate	-60.5	38.2	2	1.0	16.0	2	-60.8	38.2	2
dinoseb	-29.0	7.7	2	-7.3	3.3	2	-26.9	7.7	2
dinuron	-22.3	37.2	2	19.3	15.9	2	-27.9	37.2	2
diuron	-14.7	46.1	2	22.5	19.6	2	-21.2	46.1	2
flufenacet	-11.7	57.7	2	23.3	24.5	2	-18.4	57.7	2
imidacloprid	-40.9	38.0	2	10.2	16.0	2	-43.8	38.0	2
irgarol	-2.6	37.2	2	27.4	15.8	2	-10.5	37.2	2
isoproturon	-1.3	57.3	2	27.6	24.3	2	-9.2	57.3	2
mecoprop	-38.9	54.8	2	9.6	23.0	2	-41.7	54.8	2
methiocarb	-17.6		1	-2.1		1	-17.0		1
metolachlor	-5.7	67.3	2	25.5	28.5	2	-13.0	67.3	2
pirimicarb	-14.8	62.8	2	21.3	26.6	2	-20.9	62.8	2
quinoxyfen*									
simazine	-27.8	43.6	2	15.9	18.5	2	-32.4	43.6	2
terbuthylazine	-9.2	50.8	2	24.4	21.6	2	-16.3	50.8	2
terbutryn	-5.6	36.7	2	26.1	15.6	2	-13.1	36.7	2
thiacloprid	-19.9	45.4	2	20.1	19.2	2	-25.7	45.4	2
thiamethoxam	-54.4	38.3	2	3.5	16.0	2	-55.4	38.3	2
pharmaceuticals									
alprazolam	26.6	64.1	2	39.0	27.0	2	15.4	64.1	2
amitriptyline	-1.0	5.4	2	27.9	2.1	2	-9.1	5.4	2
azithromycin	-29.0	24.1	2	11.1	10.2	2	-32.2	24.1	2
bezafibrate	-16.1	95.3	2	20.7	40.5	2	-22.1	95.3	2
bisoprolol	-6.7	49.1	2	24.7	20.6	2	-13.8	49.1	2
carbamazepine	-7.4	56.0	2	25.2	23.8	2	-14.6	56.0	2
chloramphenicol	-10.4	58.4	2	23.2	24.7	2	-17.1	58.4	2
clorfibric acid	-20.9	0.1	2	-4.7	0.1	2	-19.5	0.1	2
clarithromycin	-7.1	58.0	2	24.5	24.4	2	-14.2	58.0	2
diazepam	-8.5	59.6	2	24.6	25.3	2	-15.6	59.6	2
diclofenac	-12.0	85.4	2	22.8	36.4	2	-18.5	85.4	2
efavirenz*									
enrofloxacin	-9.6		1	0.2		1	-9.7		1
flumequine	-10.4	12.1	2	0.0	5.4	2	-10.4	12.1	2
fluoxetine	-14.0		1	-1.1		1	-13.7		1

	$\Delta H_{288.15}$ (kJ/mol)			ΔS (J/mol/K)			$\Delta G_{288.15K}$ (kJ/mol)		
Compound	**Mean**	**SD**	**n**	**Mean**	**SD**	**n**	**Mean**	**SD**	**n**
Ifosfamide	-28.2	47.4	2	14.6	19.9	2	-32.4	47.4	2
indomethacin*									
ketoprofen	-4.7	84.3	2	25.3	35.6	2	-12.0	84.3	2
metoprolol	-3.8	35.5	2	25.7	14.9	2	-11.3	35.5	2
moxifloxacin	-12.7		1	-1.2		1	-12.4		1
nalidixic acid	-21.9	7.8	2	16.6	4.2	2	-26.7	7.8	2
nevirapine	-19.9	63.3	2	19.4	26.8	2	-25.5	63.3	2
oseltamivir ethylester	-12.3	56.8	2	21.7	24.0	2	-18.5	56.8	2
paroxetine	-15.3		1	-1.5		1	-14.9		1
propranolol	-5.6	6.4	2	26.0	2.6	2	-13.1	6.4	2
rimantadine	-32.6	10.0	2	12.7	4.2	2	-36.3	10.0	2
sarafloxacin	-15.3	5.4	2	20.1	2.0	2	-21.1	5.4	2
sulfadoxin	-10.0	39.9	2	22.4	16.1	2	-16.5	39.9	2
sulfamethazine	-25.5	35.3	2	16.2	14.6	2	-30.2	35.3	2
sulfamethoxazole	-47.1	2.8	2	6.1	2.4	2	-48.9	2.8	2
tetracycline	-9.4		1	-0.2		1	-9.3		1
trimethoprim	-18.5	35.6	2	19.8	15.2	2	-24.2	44.9	2
phthalates									
benzyl butyl phthalate	-142.9		1	-32.9		1	-133.4		1
diamyl phthalate	-130.0		1	-27.2		1	-122.1		1
dibutyl phthalate	-44.5	20.3	2	8.4	7.3	2	-31.8	-47.0	2
dicyclohexyl phthalate	-139.7	37.4	2	-31.4	14.8	2	-68.6	-130.6	2
diethyl phthalate	-75.8	46.1	2	-4.3	19.1	2	-49.2	-74.6	2
diethylheyxl phthalate	-109.7	18.2	2	-19.5	10.3	2	-51.9	-104.1	2
dihexyl phthalate	-124.7		1	-23.5		1	-117.9		1
dimethyl phthalate	-98.2	46.5	2	-13.7	20.3	2	-94.2	488.9	2
dinonyl phthalate*									
monobenzyl phthalate	-94.6		1	-9.1		1	-92.0		1
monocyclohexyl phthalate	-20.6	192.8	2	18.0	85.4	2	-25.7	184.7	2
monoethyl phthalate	-41.1		1	10.9		1	-44.2		1
monohexyl phthalate	-96.7	64.7	2	-11.9	25.4	2	-0.1	64.7	2
monomethyl phthalate	-120.6	70.1	2	-23.2	28.7	2	-0.1	70.1	2
Mono-n-pentyl phthalate	-51.6		1	5.6		1	-53.2		1
steroidal EDCs									
1,4-androstadienedione	-156.5	133.9	3	-41.4	57.8	3	-144.6	133.9	3
11-keteotiocholanolone	-80.8	34.4	3	-8.9	14.7	3	-78.3	34.4	3
11-ketotestosterone	-114.4	42.8	3	-23.2	18.3	3	-107.7	42.8	3
5α -dihydrotestosterone	-107.3	46.5	3	-20.0	19.9	3	-101.5	46.5	3
11β-hydroxyandrosterone	-137.9	77.4	3	-33.9	33.7	3	-128.2	77.4	3
17α-acetoxyprogesterone	-124.2	55.5	3	-27.0	23.9	3	-116.5	55.5	3
17α-hydroxyprogesterone	-118.6	58.8	3	-24.9	25.2	3	-111.4	58.8	3
17α-testosterone	-170.2	7.2	3	-46.1	3.9	3	-156.9	7.2	3
17α-trenbolone	-17.0	136.2	3	17.9	57.6	3	-22.2	136.2	3
17β-testosterone	-116.3	47.9	3	-23.9	20.6	3	-109.4	47.9	3
17β-trenbolone	-103.7	36.9	3	-18.5	15.7	3	-98.4	36.9	3
19-norethindron	-115.3	50.3	3	-23.4	21.9	3	-108.5	50.3	3
19-nortestosterone	-54.3	104.9	3	2.5	45.0	3	-55.1	104.9	3
α-zearalenol	-116.7	61.7	3	-23.9	26.5	3	-109.8	61.7	3
α-zeranol	-40.9	21.9	3	7.6	9.1	3	-43.1	21.9	3
β-zearalenol									
β-zeranol	-26.4	19.7	3	14.4	8.1	3	-30.6	19.7	3
androstenedione*									
androsterone*									
caproxyprogesterone	-108.0	78.3	3	-21.6	33.7	3	-101.8	78.3	3
chlorotestosteron acetate	-110.2	42.7	3	-22.2	17.7	3	-103.8	42.7	3
cortisol	-105.1	62.9	3	-18.7	27.4	3	-99.7	62.9	3
cortisone*									
dexamethasone*									
dienostrol*									

	$\Delta H_{288.15}$ (kJ/mol)			ΔS (J/mol/K)			$\Delta G_{288.15K}$ (kJ/mol)		
Compound	**Mean**	**SD**	**n**	**Mean**	**SD**	**n**	**Mean**	**SD**	**n**
diethylstilbestrol*									
epi-androsterone*									
Estrone	-131.5	70.6	3	-31.0	30.5	3	-122.6	70.6	3
ethinyl testosterone	-95.3	27.6	3	-14.8	11.9	3	-91.1	27.6	3
flugestone acetate	-84.1	26.8	3	-8.8	12.3	3	-81.5	26.8	3
fluoxymesterone	-123.4	47.0	3	-26.8	20.4	3	-115.6	47.0	3
gestodene	-193.6	46.0	3	-57.5	19.4	3	-177.0	46.0	3
medroxyprogesterone	-135.3	37.6	3	-32.0	16.4	3	-126.0	37.6	3
medroxyprogesterone acetate	-148.3	84.7	3	-37.5	36.3	3	-137.4	84.7	3
megestrol	-159.2	59.6	3	-42.5	26.1	3	-146.9	59.6	3
megestrol acetate	-143.0	96.8	3	-35.5	41.8	3	-132.8	96.8	3
mestanolone	-199.6	35.2	3	-60.5	14.8	3	-182.2	35.2	3
methylboldone	-130.5	48.9	3	-29.8	21.0	3	-121.9	48.9	3
methylprogesterone	-96.2	52.5	3	-15.8	22.4	3	-91.6	52.5	3
norethandrolone	-102.4	41.2	3	-17.8	17.7	3	-97.3	41.2	3
norgestrel	-101.6	33.6	3	-17.4	14.6	3	-96.6	33.6	3
prednisolone	-123.4	76.2	3	-26.5	32.9	3	-115.7	76.2	3
prednisone*									
progesterone	-148.8	80.9	3	-38.3	34.7	3	-137.7	80.9	3
stanozolol*									
testosterone phenylpropionate*									
testosterone acetate	-96.2	52.5	3	-15.8	22.4	3	-91.6	52.5	3
testosterone benzoate	-9.2	23.3	3	20.4	10.3	3	-15.1	23.3	3
testosterone propionate	-149.1	22.0	3	-39.4	9.9	3	-137.7	22.0	3
testosterone acetate									

APPENDIX D - ACTIVE AND PASSIVE SAMPLING BASED APPROACHES FOR MONITORING ENDOCRINE DISRUPTING COMPOUNDS IN THE BELGIAN PART OF THE NORTH SEA BETWEEN 2016 AND 2018

Table D1. Overview of the quantitative results obtained in samples from SC1, collected at open sea Zeebrugge (OZ). During the deployment and retrieval of the passive (P) samplers, active samples were taken; referred to as (D) and (R). Av and SD (n_{active}=4 and $n_{passive}$=6) indicate the average concentration and the standard deviation, respectively, both expressed in ng L^{-1}. DF represents the detection frequency (%). < MQL refers to concentrations below the method quantification limit. Blank cells refer to concentrations below the method detection limit.

	D			R			P		
	Av	SD	DF	Av	SD	DF	Av	SD	DF
Methandriol									
17α-trenbolone									
17β-trenbolone				0.3	0.1	100	1.0	1.3	67
11β-hydroxyandrosterone									
Testosterone 17β-cypionate									
17β-dihydroandrosterone									
Androsterone	2.6	0.1	75	4.4	0.1	50	2.4	0.5	100
19-nortestosterone				< MQL		75	0.8	0.7	67
1,4-Androstadienedione	1.2	0.3	75	0.2	0.1	100	1.1	0.7	100
11-ketoetiocholanolone									
Androstenedione				< MQL		100	0.4	0.2	83
Mestanolone				3.3	0.0	50	0.4	0.0	83
17α-testosterone	0.5	0.3	100	0.3	0.1	100	0.3	0.1	83
17β-testosterone									
5α-dihydrotestosterone	0.5	0.2	100	2.9	1.8	100	1.3	1.0	67
Norethindron									
Methylboldenone									
11-ketotestosterone				0.5	0.0	100	2.8	2.9	67
Formestane				0.8	0.2	100	4.1	4.1	100
Norethandrolone							0.7	0.2	67
Methyltestosterone							0.9	0.1	67
Trenbolone acetate							0.4	0.2	67
Ethynyl testosterone							0.1	0.0	67
Stanozolol									
Testosterone acetate	0.9	0.0	75	< MQL		100	1.8	1.0	67
Fluoxymesterone									
Testosterone propionate	1.3	1.1	100	0.5	0.0	75	0.3	0.2	67
Chlorotestosteron acetate									
Testosterone benzoate							0.2	0.1	67
Testosterone phenylpropionate							0.2	0.0	50
19-nortestosterone-17-decanoate									
17α-estradiol				< MQL		75			
17β-estradiol	6.8	4.0	75	6.8	4.0	100			
Estradiol-17-acetate	2.5	0.7	75	1.5	0.3	100	0.2	0.1	67
Dienoestrol				< MQL		100			
Equilin							2.2	0.2	67
Diethylstilbestrol									
Estrone				1.9	0.3	75			
17a-ethinylestradiol									
α-zearalenol									
β-zearalenol									
α-zeranol				3.1	0.4	75			
β-zeranol									
Gestodene									
Estradiol-benzoate									

	D			R			P		
	Av	SD	DF	Av	SD	DF	Av	SD	DF
5α-Pregnan-3α,20β-diol									
Norgestrel									
Dihydroprogesterone				1.9	0.0	100			
Progesterone	<MQL		100	0.8	0.0	100	0.1	0.0	50
Methylprogesterone	1.0	0.3	100	0.7	0.0	100	0.8	0.5	83
17α-hydroxyprogesterone				1.8	0.0	75			
Megestrol				2.6	0.0	100	0.6	0.2	67
Medroxyprogesterone	0.6	0.1	75	0.7	0.0	100	0.9	0.6	83
17α-acetoxyprogesterone									
Megestrol acetate	< MQL						2.0		17
Medroxyprogesterone acetate	< MQL								
Flugestone acetate							0.5	0.1	67
Caproxyprogesterone				0.9		25	1.1	0.8	83
Prednisone	9.2	8.9	100				1.5	0.6	83
Corticosterone	3.1	1.7	100	< MQL		100			
Cortisone	10.0	17.9	100	4.1	0.3	100	0.7	0.1	83
Prednisolone	6.9	1.7	100				26.5	28.7	83
Cortisol	2.8	5.6	100	2.7	0.0	50			
Tetrahydrocortisone	< MQL		100	7.7		25	20.9	27.6	67
Corticosterone acetate									
Dexamethasone							8.5	6.1	67
Prednisolone acetate	< MQL								
Cortisone acetate							6.7	5.4	83
Hydrocortisone 21-acetate							12.7	9.1	67
2-methyl phenol									
4-ethylphenol	328.0	150.0	100	407.0	440.0	100	16.7	12.6	67
4-isopropyl phenol									
4-chloro-3-methylphenol									
2,5-dichloro phenol									
3,4,6-trichlorophenol									
Bisphenol A									
Dimethyl phthalate									
Diethyl phthalate	159.0	3.0	100	27.0	3.0	100			
Dibutyl phthalate				77.0	11.0	100	12.1	5.8	83
Diamyl phthalate									
Benzyl butyl phthalate				79.0	70.0	100	5.3	1.0	83
Dicyclohexyl phthalate	67.0	93.0	100						
Dihexyl phthalate									
Dibenzyl phthalate							6.9	0.1	67
Diethylheyxl phthalate	269.0	151.0	100	298.0	145.0	100	2.2	1.4	67
Dinonyl phthalate							14.2	9.1	67
Diisodecyl phthalate				< MQL		100	8.1	1.5	67
Monomethyl phthalate	235.0	161.0	100	2545.0	226.0	100	38.4	54.2	50
Monoethyl phthalate							2.1	1.2	67
Monobutyl phthalate	176.5	23.0	100						
Mono-n-pentyl phthalate	< MQL		100	58.0	170.0	100			
Monocyclohexyl phthalate									
Monohexyl phthalate							18.7	6.2	83
Monobenzyl phthalate							2.1	1.2	67
Monoethylhexyl phthalate				740.0	391.0		37.5	21.0	67

Table D2. Overview of the quantitative results obtained in samples from SC2, collected at open sea Zeebrugge (OZ). During the deployment and retrieval of the passive (P) samplers, active samples were taken; referred to as (D) and (R). Av and SD (n_{active}=3 and $n_{passive}$=6) indicate the average concentration and the standard deviation, respectively, both expressed in ng L^{-1}. DF represents the detection frequency (%). < MQL refers to concentrations below the method quantification limit. Blank cells refer to concentrations below the method detection limit.

	D			R			P		
	Av	SD	DF	Av	SD	DF	Av	SD	DF
Methandriol							17.3	7.9	100
17α-trenbolone	1.2	0.2	67						
17β-trenbolone	0.8	0.0	100						
11β-hydroxyandrosterone	3.3	1.7	100	0.4	0.2	67			
Testosterone 17β-cypionate									
17β-dihydroandrosterone									
Androsterone	63.9	21.8	100	2.6	0.6	100			
19-nortestosterone	< MQL		33						
1,4-Androstadienedione	0.8	0.0	100	1.2	0.0	67			
11-ketoetiocholanolone	1.8	1.3	100				1.0	0.3	100
Androstenedione	0.9	0.2	100						
Mestanolone									
17α testosterone	0.3	0.2	100	0.5	0.2	100			
17β-testosterone									
5α-dihydrotestosterone	< MQL		100	0.4	0.2	100			
Norethindron	1.1	0.8	67						
Methylboldenone	1.7		33						
11-ketotestosterone	2.7	0.2	100						
Formestane	3.7	1.4	100						
Norethandrolone	1.0	0.3	100						
Methyltestosterone	1.7								
Trenbolone acetate	2.0	1.5	100						
Ethynyl testosterone	0.6	0.1	100				0.7	0.2	100
Stanozolol									
Testosterone acetate	0.6	0.4	100	0.9	0.0	100	3.9	1.6	100
Fluoxymesterone									
Testosterone propionate				1.6	0.4	100			
Chlorotestosteron acetate									
Testosterone benzoate	1.2	0.0	100						
Testosterone phenylpropionate	1.0	0.9	67				0.2	0.1	100
19-nortestosterone-17-decanoate									
17α-estradiol									
17β-estradiol				6.2	1.3	67	30.9	5.4	100
Estradiol-17-acetate				2.7	1.1	100			
Dienoestrol				< MQL		67			
Equilin							22.1	4.7	100
Diethylstilbestrol									
Estrone	1.4	0.9	67						
17α-ethinylestradiol									
α-zearalenol	3.9	1.3	100						
β-zearalenol	2.7	1.5	67						
α-zeranol	3.2	0.3	67						
β-zeranol	5.3	1.0	67						
Gestodene	1.5	0.6	67						
Estradiol-benzoate									
5α-Pregnan-3α,20β-diol									
Norgestrel	0.9	0.1	67				1.6	0.4	100
Dihydroprogesterone									
Progesterone	0.6	0.3	100				0.5	0.1	100
Methylprogesterone	0.8	0.8	67	1.1	0.3	100			
17α-hydroxyprogesterone	1.3	0.3	67						

	D			R			P		
	Av	**SD**	**DF**	**Av**	**SD**	**DF**	**Av**	**SD**	**DF**
Megestrol	7.7		33						
Medroxyprogesterone	1.9	0.5	67	0.6	0.0	67			
17α-acetoxyprogesterone	2.2	0.7	67						
Megestrol acetate									
Medroxyprogesterone acetate	2.8	1.6	67						
Flugestone acetate	2.6	0.9	67				0.4	0.2	100
Caproxyprogesterone									
Prednisone	10.9	5.2	67	7.5		33	5.7	2.4	100
Corticosterone	27.7	3.9	67	2.3	1.5	67			
Cortisone	9.5	11.7	67				4.0	2.0	100
Prednisolone	29.6	40.2	67						
Cortisol	6.5	5.9	67	3.9		33	2.7	1.5	100
Tetrahydrocortisone				2.5	0.0	100			
Corticosterone acetate	< MQL		67						
Dexamethasone	54.5	1.5	67						
Prednisolone acetate									
Cortisone acetate							174.6	24.8	100
Hydrocortisone 21-acetate	< MQL		100						
2-methyl phenol									
4-ethylphenol	433.9	250.6	67	280.1	381.2	100	23.5	7.0	100
4-isopropyl phenol							84.5	7.3	67
4-chloro-3-methylphenol									
2,5-dichloro phenol									
3,4,6-trichlorophenol									
Bisphenol A							21.8	4.7	67
Dimethyl phthalate									
Diethyl phthalate				923.2	837.4	67			
Dibutyl phthalate	1866.7	411.6	100	542.0	805.6	100	14.8	3.3	100
Diamyl phthalate									
Benzyl butyl phthalate	56.6	23.8	100	79.4	69.8	100	5.7	1.4	100
Dicyclohexyl phthalate	132.4		33						
Dihexyl phthalate	152.1	183.8	67						
Dibenzyl phthalate									
Diethylheyxl phthalate	269.1	150.5	67	298.3	144.7	100	0.5	0.2	50
Dinonyl phthalate									
Diisodecyl phthalate							9.7	1.0	83
Monomethyl phthalate	2329.8	1583.4	67	2542.4	222.7	67			
Monoethyl phthalate									
Monobutyl phthalate				26.0	10.0	100			
Mono-n-pentyl phthalate	577.2	320.3	67	170.2	137.6	67			
Monocyclohexyl phthalate									
Monohexyl phthalate							15.1	3.8	67
Monobenzyl phthalate	56.7	17.4	67						
Monoethylhexyl phthalate				739.9	391.1	100	14.5	5.6	100

Table D3. Overview of the quantitative results obtained in samples from SC3, collected at open sea Zeebrugge (OZ). During the deployment and retrieval of the passive (P) samplers, active samples were taken; referred to as (D) and (R). Av and SD (n_{active}=3 and $n_{passive}$=6) ndicate the average concentration and the standard deviation, respectively, both expressed in ng L^{-1}. DF represents the detection frequency (%). < MQL refers to concentrations below the method quantification limit. Blank cells refer to concentrations below the method detection limit.

	D			R			P		
	Av	SD	DF	Av	SD	DF	Av	SD	DF
Methandriol									
17α-trenbolone	0.8	0.2	67	0.8	0.3	67			
17β-trenbolone	1.0	0.6	67	1.5	0.2	67			
11β-hydroxyandrosterone	0.9	0.4	100	0.6	0.1	67			
Testosterone 17β-cypionate									
17β-dihydroandrosterone	1.7	0.4	67						
Androsterone									
19-nortestosterone	4.5	0.5	100	2.6	0.7	100			
1,4-Androstadienedione									
11-ketoetiocholanolone							< MQL		100
Androstenedione	< MQL		100	0.6	0.3	67			
Mestanolone									
17α-testosterone	2.8	0.6	100	2.3	0.4	100			
17β-testosterone									
5α-dihydrotestosterone	< MQL		100	< MQL		100			
Norethindron				4.4	2.7	100			
Methylboldenone	2.3	0.2	67						
11-ketotestosterone	0.2	0.1	100						
Formestane				22.5	3.6	100			
Norethandrolone									
Methyltestosterone	0.9	0.2	67	0.6	0.1	67			
Trenbolone acetate									
Ethynyl testosterone	0.6	0.0	67	0.8	0.1	67			
Stanozolol									
Testosterone acetate				1.5	0.6	67			
Fluoxymesterone									
Testosterone propionate	0.7	0.2	67	1.3	0.4	67	1.1	0.1	67
Chlorotestosteron acetate	0.6	0.0	67						
Testosterone benzoate	1.3	0.1	67	1.3	0.1	67	0.1	0.0	67
Testosterone phenylpropionate	0.8	0.5	100	18.8	19.9	100	0.5	0.7	67
19-nortestosterone-17-decanoate									
17α-estradiol	< MQL		100						
17β-estradiol	8.4	2.7	100	85.0	5.2	100			
Estradiol-17-acetate	1.5	0.6	67	21.7	4.6	100			
Dienoestrol				< MQL		67			
Equilin							0.8	0.3	50
Diethylstilbestrol									
Estrone									
17α-ethinylestradiol				< MQL		67			
α-zearalenol									
β-zearalenol									
α-zeranol	< MQL		67	< MQL		67			
β-zeranol				< MQL		67			
Gestodene									
Estradiol-benzoate	< MQL		100						
5α-Pregnan-3α,20β-diol									
Norgestrel	0.5	0.1	67						
Dihydroprogesterone	0.8	0.0	100	0.8	0.0	67			
Progesterone	7.9	2.5	100	7.4	1.6	100	0.1	0.0	67

	D			R			P		
	Av	SD	DF	Av	SD	DF	Av	SD	DF
Methylprogesterone				1.2	0.2	67	0.3	0.1	67
17α-hydroxyprogesterone				11.2	2.3	100	1.4	0.2	67
Megestrol	1.4	0.1	100	1.4	0.1	100			
Medroxyprogesterone	0.3	0.2	100	1.2	0.3	67	0.1	0.1	50
17α-acetoxyprogesterone	1.8	0.1	100	2.5	0.8	100	0.4	0.0	67
Megestrol acetate	0.9	0.1	67	0.9	0.6	100			
Medroxyprogesterone acetate				0.7	0.4	100			
Flugestone acetate	1.3	0.6	67	1.5	0.5	100			
Caproxyprogesterone	1.2	0.1	67	1.8	0.1	67	0.3	0.0	83
Prednisone				15.2	3.5	67	1.6	0.4	83
Corticosterone	5.8	0.8	67	3.7	1.6	67			
Cortisone	2.7	1.0	67	< MQL		67	1.4		17
Prednisolone				9.0	0.4	67	1.3	0.1	83
Cortisol				5.9	1.8	67	0.1	0.0	83
Tetrahydrocortisone	< MQL		67	< MQL		100			
Corticosterone acetate	< MQL		67	< MQL		67			
Dexamethasone	9.7	0.3	67	9.6	0.3	67	2.4	0.2	50
Prednisolone acetate									
Cortisone acetate							229.3	59.9	67
Hydrocortisone 21-acetate									
2-methyl phenol									
4-ethylphenol	2064.6	1192.5	67				79.6	43.7	67
4-isopropyl phenol							94.0	2.7	50
4-chloro-3-methylphenol									
2,5-dichloro phenol									
3,4,6-trichlorophenol									
Bisphenol A							18.4	2.1	67
Dimethyl phthalate									
Diethyl phthalate	478.2	463.5	67						
Dibutyl phthalate	5296.5	4839.7	67	112.3	79.9	67	43.1	34.4	83
Diamyl phthalate	< MQL		100						
Benzyl butyl phthalate	< MQL		100	37.1	8.5	67	5.9	1.4	83
Dicyclohexyl phthalate	< MQL		100	24.5		33			
Dihexyl phthalate									
Dibenzyl phthalate	< MQL		100						
Diethylheyxl phthalate	190.6	130.1	67				3.8	2.4	100
Dinonyl phthalate							35.9	38.2	100
Diisodecyl phthalate							18.1	6.7	83
Monomethyl phthalate									
Monoethyl phthalate									
Monobutyl phthalate	458.8	560.0	67						
Mono-n-pentyl phthalate							4.0	2.1	50
Monocyclohexyl phthalate	89.8	5.5	100						
Monohexyl phthalate							23.1	13.0	50
Monobenzyl phthalate							3.1	1.2	50
Monoethylhexyl phthalate							8.3	1.0	50

Table D4. Overview of the quantitative results obtained in samples from SC4, collected at open sea Zeebrugge (OZ). D represents the active samling during deployement. Av and SD (n_{active}=3 and $n_{passive}$=6) indicate the average concentration and the standard deviation, respectively, both expressed in ng L^{-1}. DF represents the detection frequency (%). < MQL refers to concentrations below the method quantification limit. Blank cells refer to concentrations below the method detection limit.

	D		
	Av	SD	DF
Methandriol			
17α-trenbolone			
17β-trenbolone	< MQL		100
11β-hydroxyandrosterone			
Testosterone 17β-cypionate			
17β-dihydroandrosterone			
Androsterone			
19-nortestosterone			
1,4-Androstadienedione			
11-ketoetiocholanolone	< MQL		67
Androstenedione			
Mestanolone			
17α-testosterone			
17β-testosterone	0.3	0.0	100
5α-dihydrotestosterone	0.2	0.1	100
Norethindron			
Methylboldenone	< MQL		100
11-ketotestosterone			
Formestane			
Norethandrolone			
Methyltestosterone	0.4	0.0	67
Trenbolone acetate			
Ethynyl testosterone			
Stanozolol			
Testosterone acetate			
Fluoxymesterone			
Testosterone propionate			
Chlorotestosteron acetate			
Testosterone benzoate			
Testosterone phenylpropionate			
19-nortestosterone-17-decanoate			
17α-estradiol			
17β-estradiol			
Estradiol-17-acetate	3.9	4.1	67
Dienoestrol			
Equilin			
Diethylstilbestrol			
Estrone			
17α-ethinylestradiol			
α-zearalenol			
β-zearalenol			
α-zeranol			
β-zeranol			
Gestodene			
Estradiol-benzoate			
5α-Pregnan-3α,20β-diol			
Norgestrel	2.9	0.2	67
Dihydroprogesterone			
Progesterone			
Methylprogesterone			
17α-hydroxyprogesterone	0.3	0.0	67
Megestrol			
Medroxyprogesterone	< MQL		67
17α-acetoxyprogesterone			

	D Av	SD	DF
Megestrol acetate			
Medroxyprogesterone acetate			
Flugestone acetate			
Caproxyprogesterone			
Prednisone			
Corticosterone			
Cortisone	< MQL		67
Prednisolone			
Cortisol			
Tetrahydrocortisone			
Corticosterone acetate	125.4		33
Dexamethasone	3.5	0.2	67
Prednisolone acetate			
Cortisone acetate			
Hydrocortisone 21-acetate			
2-methyl phenol			
4-ethylphenol	1233.1	4494.6	100
4-isopropyl phenol	392.6	3.3	100
4-chloro-3-methylphenol	< MQL		67
2,5-dichloro phenol			
3,4,6-trichlorophenol			
Bisphenol A	961.3	330.2	67
Dimethyl phthalate	< MQL		100
Diethyl phthalate			
Dibutyl phthalate	240.5	132.5	100
Diamyl phthalate	17.3	8.2	100
Benzyl butyl phthalate	76.6	1.6	67
Dicyclohexyl phthalate	< MQL		
Dihexyl phthalate	31.1	19.6	100
Dibenzyl phthalate			
Diethylheyxl phthalate			
Dinonyl phthalate			
Diisodecyl phthalate			
Monomethyl phthalate	488.2	238.3	67
Monoethyl phthalate			
Monobutyl phthalate	132.5	59.0	100
Mono-n-pentyl phthalate	59.1	28.1	100
Monocyclohexyl phthalate			
Monohexyl phthalate			
Monobenzyl phthalate	61.2	5.4	67
Monoethylhexyl phthalate			

Table D5. Overview of the quantitative results obtained in samples from SC1, collected at harbour of Oostende (HO). During the deployment and retrieval of the passive (P) samplers, active samples were taken; referred to as (D) and (R). Av and SD (n_{active}=4 and $n_{passive}$=6) indicate the average concentration and the standard deviation, respectively, both expressed in ng L^{-1}. DF represents the detection frequency (%). < MQL refers to concentrations below the method quantification limit. Blank cells refer to concentrations below the method detection limit.

	D			R			P		
	Av	SD	DF	Av	SD	DF	Av	SD	DF
Methandriol									
17α-trenbolone									
17β-trenbolone				0.9	0.5	100	0.1	0.0	83
11β-hydroxyandrosterone									
Testosterone 17β-cypionate									
17β-dihydroandrosterone									
Androsterone	3.6	0.1	50						
19-nortestosterone				< MQL		100			
1,4-Androstadienedione	1.6	0.3	75	0.8	0.7	100	0.4	0.0	67
11-ketoetiocholanolone									
Androstenedione				< MQL		100			
Mestanolone							0.4	0.1	50
17α-testosterone	1.1	0.3	100	0.3	0.1	100	0.2	0.1	50
17β-testosterone									
5α-dihydrotestosterone	1.9	0.5	100	< MQL		100	0.8	0.2	33
Norethindron									
Methylboldenone									
11-ketotestosterone				2.3	0.3	50	1.0	1.1	50
Formestane				2.0	0.6	100	0.5	0.2	50
Norethandrolone							0.6		17
Methyltestosterone									
Trenbolone acetate							0.2		17
Ethynyl testosterone	0.6	0.0	100				0.0	0.0	67
Stanozolol									
Testosterone acetate	1.1	0.1	75	< MQL		50	0.9	0.3	67
Fluoxymesterone									
Testosterone propionate	2.7	1.1	100				0.5	0.3	67
Chlorotestosteron acetate									
Testosterone benzoate									
Testosterone phenylpropionate									
19-nortestosterone-17-decanoate									
17α-estradiol				< MQL		100			
17β-estradiol	9.7	4.0	100	6.4	0.1	75	0.6	0.1	83
Estradiol-17-acetate	10.4	6.9	100				0.3	0.1	33
Dienoestrol									
Equilin									
Diethylstilbestrol									
Estrone				1.9	0.2	100	0.3	0.0	67
17α-ethinylestradiol									
α-zearalenol									
β-zearalenol									
α-zeranol				3.9	1.1	50			
β-zeranol									
Gestodene									
Estradiol-benzoate									
5α-Pregnan-3α,20β-diol									
Norgestrel									
Dihydroprogesterone									
Progesterone	< MQL		100				0.2	0.1	67
Methylprogesterone	0.8	0.5	100	0.7	0.0	50	0.1	0.0	67
17α-hydroxyprogesterone									
Megestrol							0.4	0.0	67
Medroxyprogesterone	0.8	0.3	75	0.7	0.0	50	0.2	0.3	67

	D			R			P		
	Av	SD	DF	Av	SD	DF	Av	SD	DF
17α-acetoxyprogesterone	1.2	0.1	100				0.5	0.3	67
Megestrol acetate	< MQL		25						
Medroxyprogesterone acetate	1.1	1.0	75						
Flugestone acetate									
Caproxyprogesterone				1.1	0.2	100	0.2	0.0	33
Prednisone	39.1	8.9	50				7.4	1.4	10
Corticosterone	4.6	1.7	75	< MQL					
Cortisone	28.2	17.9	50	5.5	0.9	100	2.1	1.8	67
Prednisolone				7.7	0.9	100	4.1	0.2	5
Cortisol	7.5	5.6	100	3.1	0.1	75			
Tetrahydrocortisone	< MQL		100	8.8	0.7	75	1.7	0.4	67
Corticosterone acetate									
Dexamethasone							2.0	0.8	33
Prednisolone acetate	< MQL		100						
Cortisone acetate	< MQL		100						
Hydrocortisone 21-acetate				5.0	0.0	75			
2-methyl phenol	215.4	47.8	100	6502.6	1791.7	75			
4-ethylphenol	2508.0	243.0	100	468.7	158.8	75	82.6	20.5	67
4-isopropyl phenol							81.3	0.3	33
4-chloro-3-methylphenol									
2,5-dichloro phenol									
3,4,6-trichlorophenol									
Bisphenol A							42.7	14.8	83
Dimethyl phthalate									
Diethyl phthalate	235.0	111.0	100	753.0	95.0	75			
Dibutyl phthalate	2645.0	250.0	100	1502.0	401.0	75	23.0	15.1	83
Diamyl phthalate				< MQL		75			
Benzyl butyl phthalate				343.0	283.0	75	8.8	2.9	83
Dicyclohexyl phthalate							8.6	1.2	67
Dihexyl phthalate									
Dibenzyl phthalate							6.8	0.8	33
Diethylheyxl phthalate	100.1	70.5	100	704.4	246.7	75	2.6	2.2	83
Dinonyl phthalate							16.2	14.2	83
Diisodecyl phthalate				< MQL		75	41.6	44.3	50
Monomethyl phthalate									
Monoethyl phthalate							1.1	0.4	33
Monobutyl phthalate	165.0	28.0		192.3	7.5	75			
Mono-n-pentyl phthalate				138.0	100.0	75	2.6		17
Monocyclohexyl phthalate									
Monohexyl phthalate	53.1	30.2	100				13.7	4.3	67
Monobenzyl phthalate				58.1	1.0	50	< MQL		33
Monoethylhexyl phthalate	399.0	98.0	100	656.3	123.4	75	27.4	7.9	67

Table D6. Overview of the quantitative results obtained in samples from SC2, collected at harbour of Oostende (HO). During the deployment and retrieval of the passive (P) samplers, active samples were taken; referred to as (D) and (R). Av and SD (n_{active}=3 and $n_{passive}$=6) indicate the average concentration and the standard deviation, respectively, both expressed in ng L^{-1}. DF represents the detection frequency (%). < MQL refers to concentrations below the method quantification limit. Blank cells refer to concentrations below the method detection limit.

	D			R			P		
	Av	SD	DF	Av	SD	DF	Av	SD	DF
Methandriol							27.0	7.7	100
17α-trenbolone									
17β-trenbolone	0.8	0.3	67	0.7	0.1	100			
11β-hydroxyandrosterone									
Testosterone 17β-cypionate									
17β-dihydroandrosterone									
Androsterone				83.2	38.3	100			
19-nortestosterone	3.9	1.1	100						
1,4-Androstadienedione	2.7	1.6	100	1.4	0.4	100	5.1	2.4	83
11-ketoetiocholanolone	2.2	1.2	67	1.5	0.7	67	1.3	0.9	67
Androstenedione	1.7	0.3	67	0.8	0.1	67			
Mestanolone				14.2		33			
17α-testosterone	1.0	0.3	67	< MQL		67			
17β-testosterone	1.1	1.4	67						
5α-dihydrotestosterone	< MQL	0.0	100	< MQL		100			
Norethindron	1.2	0.2	100	< MQL		67			
Methylboldenone				1.0	0.7	67			
11-ketotestosterone	6.5	5.0	10	0.4	0.0	67			
Formestane	7.9	1.1	67	2.9	0.7	67			
Norethandrolone									
Methyltestosterone									
Trenbolone acetate	3.6		33						
Ethynyl testosterone	1.3	0.4	100	0.5	0.1	100			
Stanozolol									
Testosterone acetate	2.9	0.1	67	< MQL		67			
Fluoxymesterone									
Testosterone propionate	0.7	0.5	100!	0.6	0.0	67			
Chlorotestosteron acetate									
Testosterone benzoate							0.3	0.3	50
Testosterone phenylpropionate	2.8	0.4	100	3.8	2.8	100	0.2	0.1	50
19-nortestosterone-17-decanoate							2.6	2.0	33
17α-estradiol									
17β-estradiol							50.3	7.3	83
Estradiol-17-acetate									
Dienoestrol									
Equilin	2.8	0.2	67						
Diethylstilbestrol	2.0	1.9	67						
Estrone	0.3	0.0	67	0.9		33			
17α-ethinylestradiol									
α-zearalenol				3.0	0.4	67			
β-zearalenol	2.7	0.6	67	5.0	1.8	67			
α-zeranol	1.0		33	1.6		33			
β-zeranol	3.4	0.1	67	3.7	0.5	67			
Gestodene	1.0	0.1	67						
Estradiol-benzoate									
5α-Pregnan-3α,20β-diol									
Norgestrel				0.7		33			
Dihydroprogesterone									
Progesterone	0.4	0.2	100	< MQL		100	0.3	0.2	100
Methylprogesterone	1.6	1.4	100						
17α-hydroxyprogesterone	15.3	5.0	100	2.2	0.0	67			
Megestrol									
Medroxyprogesterone	0.8	0.7	67	< MQL		67			

	D			R			P		
	Av	SD	DF	Av	SD	DF	Av	SD	DF
17α-acetoxyprogesterone	< MQL		100				0.7	0.7	100
Megestrol acetate									
Medroxyprogesterone acetate	1.0	0.4	100	2.6	1.6	100			
Flugestone acetate	3.7	1.4	67	2.6	0.0	100	0.4	0.2	67
Caproxyprogesterone									
Prednisone							5.7	1.3	100
Corticosterone									
Cortisone	3.2	2.0	100				9.3	3.9	100
Prednisolone	4.5	0.2	67	< MQL		67	4.0	1.2	100
Cortisol	1.3	0.1	67	1.5	0.3	100	1.1	0.5	100
Tetrahydrocortisone									
Corticosterone acetate	< MQL		100	< MQL		67			
Dexamethasone							2.3	0.7	67
Prednisolone acetate									
Cortisone acetate							56.5	34.7	100
Hydrocortisone 21-acetate	< MQL		100						
2-methyl phenol	309.2	103.5	67	153.3	84.8	67			
4-ethylphenol	2525.5	174.3	100	33.9	5.8	67	20.0	13.0	83
4-isopropyl phenol									
4-chloro-3-methylphenol									
2,5-dichloro phenol									
3,4,6-trichlorophenol									
Bisphenol A							18.4	2.7	67
Dimethyl phthalate							9.9	1.3	67
Diethyl phthalate	235.3	111.4	100	3772.7	5259.3	67			
Dibutyl phthalate	2644.7	250.1	100	1433.0	1021.6	67	16.8	6.3	100
Diamyl phthalate									
Benzyl butyl phthalate				137.1	160.0	100	5.4	1.9	67
Dicyclohexyl phthalate									
Dihexyl phthalate	34.1	5.2	67	21.5	1.9	67			
Dibenzyl phthalate							6.9	0.1	67
Diethylheyxl phthalate	205.0	86.7	100	443.2	58.1	67	1.9	0.5	50
Dinonyl phthalate	446.3	374.9	100				11.8	3.4	50
Diisodecyl phthalate	440.8	446.3	100				14.4	5.5	67
Monomethyl phthalate	157.4	48.9	100						
Monoethyl phthalate									
Monobutyl phthalate			100	480.6	538.0	67			
Mono-n-pentyl phthalate	248.2		100	184.0		33			
Monocyclohexyl phthalate	157.7	128.2							
Monohexyl phthalate	138.2								
Monobenzyl phthalate	448.9	308.0	100						
Monoethylhexyl phthalate				1545.6	220.1	67	22.4	3.1	83

Table D7. Overview of the quantitative results obtained in samples from SC3, collected at harbour of Oostende (HO). During the deployment and retrieval of the passive (P) samplers, active samples were taken; referred to as (D) and (R). Av and SD (n_{active}=3 and $n_{passive}$=6) indicate the average concentration and the standard deviation, respectively, both expressed in ng L^{-1}. DF represents the detection frequency (%). < MQL refers to concentrations below the method quantification limit. Blank cells refer to concentrations below the method detection limit.

	D			R			P		
	Av	SD	DF	Av	SD	DF	Av	SD	DF
Methandriol									
17α-trenbolone				1.7	0.6	67			
17β-trenbolone				3.0	2.7	67			
11β-hydroxyandrosterone				1.7	1.1	100			
Testosterone 17β-cypionate	4.8	5.3	67						
17β-dihydroandrosterone									
Androsterone	2.5	1.9	100						
19-nortestosterone									
1,4-Androstadienedione	1.5	0.2	67						
11-ketoetiocholanolone							0.5	0.5	67
Androstenedione				3.4	1.3	67			
Mestanolone	7.1	1.0	100						
17α-testosterone	2.1	0.3	100	3.4	0.4	67			
17β-testosterone				3.5	1.4	67			
5α-dihydrotestosterone	1.6	2.5	100	< MQL		100			
Norethindron	7.4	5.0	67	13.6	3.9	67			
Methylboldenone	2.2		33	2.3	1.0	67			
11-ketotestosterone				2.1	1.0	67			
Formestane									
Norethandrolone									
Methyltestosterone				5.8	0.1	67			
Trenbolone acetate				1.1		33			
Ethynyl testosterone	0.9	0.3	100	2.0	0.6	67			
Stanozolol									
Testosterone acetate	2.8		33	2.5	1.2	67			
Fluoxymesterone	< MQL		100	< MQL		100			
Testosterone propionate	1.6	1.6	67	1.9	0.2	67	0.5	0.5	50
Chlorotestosteron acetate				0.8		33			
Testosterone benzoate	1.2	0.7	67	2.4	1.6	67	0.2	0.0	67
Testosterone phenylpropionate	21.9	12.9	67	64.1	28.5	100	2.5	1.3	67
19-nortestosterone-17-decanoate									
17α-estradiol	5.9		33						
17β-estradiol	6.5	1.9	67	29.3	4.9	67			
Estradiol-17-acetate	3.2	1.3	33	34.9	0.1	67			
Dienoestrol	4.3	2.6	67	16.0	3.7	67			
Equilin				2.3	3.1	67	9.0	1.9	50
Diethylstilbestrol				3.7		33			
Estrone				< MQL		67			
17α-ethinylestradiol									
α-zearalenol	< MQL		67						
β-zearalenol									
α-zeranol	< MQL		67						
β-zeranol	< MQL		67	1.5	1.6	67			
Gestodene									
Estradiol-benzoate							0.6	0.5	50
5α-Pregnan-3α,20β-diol				3.9		33			
Norgestrel				0.5	0.1	67			
Dihydroprogesterone	1.2	0.4	100	2.3		33			
Progesterone	14.4	3.1	100	14.8	1.7	100	0.6	0.7	83
Methylprogesterone	1.9	0.1	100	1.4		33	0.2	0.0	83
17α-hydroxyprogesterone	11.1	1.5	100	28.0	4.2	100	4.9	0.9	83
Megestrol				1.9	0.2	67			
Medroxyprogesterone									

	D			R			P		
	Av	SD	DF	Av	SD	DF	Av	SD	DF
17α-acetoxyprogesterone	3.0	1.6	67	2.0	0.3	67			
Megestrol acetate									
Medroxyprogesterone acetate	2.8	1.9	67	2.9	1.1	67			
Flugestone acetate	1.3	0.0	67	1.5	0.1	67			
Caproxyprogesterone	1.2	0.0	67	2.2	0.7	100			
Prednisone				24.4	9.9	100	3.5	2.0	83
Corticosterone	4.2		33						
Cortisone	7.2	4.2	100	28.6	12.1	67	5.0	1.8	67
Prednisolone	12.2		33	59.1		33	4.4	1.4	67
Cortisol							1.4	0.8	67
Tetrahydrocortisone	< MQL		67	3.8	1.9	67			
Corticosterone acetate				< MQL		67			
Dexamethasone	10.7	1.3	67	12.0	1.5	100	3.2	1.0	67
Prednisolone acetate									
Cortisone acetate							111.4	53.7	83
Hydrocortisone 21-acetate									
2-methyl phenol	124.3	98.9	67						
4-ethylphenol	1486.6	574.2	67	619.6	620.9	100	189.9	269.5	67
4-isopropyl phenol							91.2	12.5	50
4-chloro-3-methylphenol							28.3	0.1	67
2,5-dichloro phenol									
3,4,6-trichlorophenol									
Bisphenol A				< MQL		67	55.0	23.0	33
Dimethyl phthalate							10.3	0.8	33
Diethyl phthalate	371.9	353.4	67						
Dibutyl phthalate	416.6	54.8	67	439.9	244.8	100	29.2	20.8	67
Diamyl phthalate	< MQL		100				9.5		17
Benzyl butyl phthalate				128.3	74.5	100	5.7	1.3	50
Dicyclohexyl phthalate	20.1		33	53.5	54.4	100	8.5		17
Dihexyl phthalate									
Dibenzyl phthalate	< MQL		33	3.0			6.9	0.1	67
Diethylheyxl phthalate	368.8	393.7	67	197.0	142.5	100	1.3	0.2	50
Dinonyl phthalate				242.5	131.6	100	8.4	1.3	50
Diisodecyl phthalate							53.8	23.5	50
Monomethyl phthalate							27.1	33.8	67
Monoethyl phthalate							1.6	0.7	50
Monobutyl phthalate	382.1	362.1	67						
Mono-n-pentyl phthalate									
Monocyclohexyl phthalate									
Monohexyl phthalate							7.6	3.2	83
Monobenzyl phthalate									
Monoethylhexyl phthalate									

Table D8. Overview of the quantitative results obtained in samples from SC4, collected at harbour of Oostende (HO). During the deployment and retrieval of the passive (P) samplers, active samples were taken; referred to as (D) and (R). Av and SD (n_{active}=3 and $n_{passive}$=6) indicate the average concentration and the standard deviation, respectively, both expressed in ng L^{-1}. DF represents the detection frequency (%). < MQL refers to concentrations below the method quantification limit. Blank cells refer to concentrations below the method detection limit.

	D			R			P		
	Av	SD	DF	Av	SD	DF	Av	SD	DF
Methandriol							41.3	9.0	100
17α-trenbolone	< MQL		100						
17β-trenbolone	0.5	0.3	67						
11β-hydroxyandrosterone									
Testosterone 17β-cypionate									
17β-dihydroandrosterone									
Androsterone									
19-nortestosterone				0.6	0.3	67			
1,4-Androstadienedione				0.5	0.1	100			
11-ketoetiocholanolone	0.6	0.3	100	0.4	0.0	67			
Androstenedione									
Mestanolone				< MQL		67			
17α-testosterone	0.8	0.4	67						
17β-testosterone	0.4	0.1	100						
5α-dihydrotestosterone	0.3	0.2	100	< MQL		100	13.2	2.9	100
Norethindron									
Methylboldenone	< MQL		67						
11-ketotestosterone	< MQL		67	0.3	0.2	67			
Formestane									
Norethandrolone	0.6		33						
Methyltestosterone	0.7	0.1	100	0.8	0.1	67			
Trenbolone acetate	< MQL		100						
Ethynyl testosterone	0.8	0.0	100	0.2	0.1	100			
Stanozolol									
Testosterone acetate									
Fluoxymesterone	< MQL		67						
Testosterone propionate	0.4	0.1	100						
Chlorotestosteron acetate									
Testosterone benzoate	< MQL		67				0.1	0.0	50
Testosterone phenylpropionate	< MQL		67				0.1	0.0	67
19-nortestosterone-17-decanoate							1.1		17
17α-estradiol									
17β-estradiol	< MQL		67				35.2	6.9	100
Estradiol-17-acetate									
Dienoestrol									
Equilin									
Diethylstilbestrol				0.6	0.1	100			
Estrone				0.5	0.3	100			
17α-ethinylestradiol									
α-zearalenol	14.7	4.4	100						
β-zearalenol	14.8	4.1	100						
α-zeranol	< MQL		67						
β-zeranol				< MQL		67			
Gestodene	3.4	2.3	100	0.7	0.1	100			
Estradiol-benzoate									
5α-Pregnan-3α,20β-diol									
Norgestrel	3.1	0.2	100						
Dihydroprogesterone				0.4	0.0	67			
Progesterone							0.8	0.6	100
Methylprogesterone									
17α-hydroxyprogesterone	0.4	0.1	100	< MQL		100			
Megestrol									
Medroxyprogesterone				< MQL		33			

	D			R			P		
	Av	**SD**	**DF**	**Av**	**SD**	**DF**	**Av**	**SD**	**DF**
17α-acetoxyprogesterone	< MQL		67	0.4	0.2	67			
Megestrol acetate							0.2	0.0	50
Medroxyprogesterone acetate							0.3	0.0	33
Flugestone acetate									
Caproxyprogesterone							0.3	0.1	50
Prednisone							4.1	1.1	100
Corticosterone									
Cortisone	< MQL		67				4.4	1.9	100
Prednisolone				< MQL		67	3.1	1.6	83
Cortisol				< MQL		67	1.0	0.8	67
Tetrahydrocortisone				< MQL		67	2.0	1.2	83
Corticosterone acetate									
Dexamethasone	5.5	1.3	67				2.9	0.5	50
Prednisolone acetate									
Cortisone acetate							224.2	46.5	100
Hydrocortisone 21-acetate									
2-methyl phenol									
4-ethylphenol	782.1	329.6	100	213.4	86.1	100	122.0	93.0	100
4-isopropyl phenol	244.1	21.2	67						
4-chloro-3-methylphenol	< MQL		67				28.7	0.4	67
2,5-dichloro phenol									
3,4,6-trichlorophenol				68.4	3.5	100			
Bisphenol A	1007.8	810.2	100	263.2	33.8	100	22.6	5.9	83
Dimethyl phthalate									
Diethyl phthalate				< MQL		33			
Dibutyl phthalate	861.6	657.2	100	39.9	2.3	100	10.8	8.0	100
Diamyl phthalate	< MQL		67				9.4	0.0	50
Benzyl butyl phthalate	102.9	113.7	100	47.1	5.4	100	5.0	0.2	33
Dicyclohexyl phthalate	43.4	27.8	100	78.8	24.6	67	8.6	0.1	100
Dihexyl phthalate	16.2	8.7	67						
Dibenzyl phthalate									
Diethylheyxl phthalate	307.1	136.8	100	206.7	78.5	67			
Dinonyl phthalate	336.9	203.1	100						
Diisodecyl phthalate	440.0	261.7	100				25.4	15.3	33
Monomethyl phthalate									
Monoethyl phthalate				218.2	144.3	100			
Monobutyl phthalate	29.9		33						
Mono-n-pentyl phthalate	242.2	123.1	100	529.9	166.4	100			
Monocyclohexyl phthalate	300.0	228.8	100	189.2	78.7	100			
Monohexyl phthalate	332.8	51.7	100	383.7	202.3	100	6.9	1.2	100
Monobenzyl phthalate				214.1	59.4	100			
Monoethylhexyl phthalate	549.7	393.9	100	232.1	94.0	67			

Table D9. Overview of the quantitative results obtained in samples from SC1, collected at harbour of Zeebrugge (HZ). During the deployment and retrieval of the passive (P) samplers, active samples were taken; referred to as (D) and (R). Av and SD (n_{active}=4 and $n_{passive}$=6) indicate the average concentration and the standard deviation, respectively, both expressed in ng L^{-1}. DF represents the detection frequency (%). < MQL refers to concentrations below the method quantification limit. Blank cells refer to concentrations below the method detection limit.

	D			R			P		
	Av	SD	DF	Av	SD	DF	Av	SD	DF
Methandriol									
17α-trenbolone									
17β-trenbolone				0.6	0.2	100	0.1	0.0	83
11β-hydroxyandrosterone	0.6	0.1	100						
Testosterone cypionate	1.3	0.4	75						
17β-dihydroandrosterone									
Androsterone	3.3	1.4	100				1.9	0.0	50
19-nortestosterone				< MQL		100			
1,4-Androstadienedione	1.5	0.5	100	0.3	0.1	100	0.2	0.1	50
11-ketoetiocholanolone									
Androstenedione				< MQL		100	0.1	0.0	50
Mestanolone	< MQL		100	3.4	0.0	50	0.4	0.0	50
17α-testosterone	0.9	0.3	100	0.3	0.0	100	0.2	0.0	83
17β-testosterone									
5α-dihydrotestosterone	0.9	0.2	100	3.8	0.0	100	0.6	0.0	50
Norethindron									
Methylboldenone									
11-ketotestosterone	2.1	0.6	100	0.5	0.1	100	0.4	0.1	83
Formestane				0.7	0.1	100	0.5	0.1	83
Norethandrolone							0.5		17
Methyltestosterone	0.3	0.1	100						
Trenbolone acetate									
Ethynyl testosterone				0.3	0.0	75	0.0	0.0	33
Stanozolol									
Testosterone acetate	1.0	0.1	100	< MQL		100			
Fluoxymesterone									
Testosterone propionate	1.3	0.1	100	0.5	0.0	100	0.4	0.1	33
Chlorotestosteron acetate									
Testosterone benzoate	1.5	0.2	75						
Testosterone phenylpropionate									
19-nortestosterone-17-decanoate									
17α-estradiol				< MQL		100			
17β-estradiol	6.8	4.0	100	7.6	0.8	75	1.2	0.5	67
Estradiol-17-acetate	3.0	1.7	100	2.0	0.7	100			
Dienoestrol				< MQL		100	0.5		17
Equilin				< MQL					
Diethylstilbestrol									
Estrone				2.0	0.5	100	0.4	0.3	67
17α-ethinylestradiol									
α-zearalenol									
β-zearalenol									
α-zeranol							0.2	0.3	67
β-zeranol									
Gestodene				1.3	0.0	100			
Estradiol-benzoate	3.5	0.2	100						
5α-Pregnan-3α,20β-diol									
Norgestrel	0.4	0.0	100	1.7	0.0	75			
Dihydroprogesterone				1.9	0.0	100	0.4	0.0	83
Progesterone	< MQL		100	0.7	0.1	75	0.1	0.0	33
Methylprogesterone	0.4	0.3	100	0.7	0.0	100	0.1	0.0	83
17α-hydroxyprogesterone				1.8	0.0	50	0.5	0.1	33
Megestrol							0.4	0.0	83
Medroxyprogesterone	0.7	0.2	100	0.7	0.0	100	0.1	0.0	83

	D			R			P		
	Av	SD	DF	Av	SD	DF	Av	SD	DF
17a-acetoxyprogesterone				2.1	0.0	100	0.5	0.0	83
Megestrol acetate	< MQL		75						
Medroxyprogesterone	< MQL		75						
Flugestone acetate						25			
Caproxyprogesterone				0.9	0.0	50	0.2	0.0	50
Prednisone							2.2		17
Corticosterone	2.6	0.5	100	< MQL		75	0.5	0.0	33
Cortisone	7.0	3.2	100	4.9	0.5	100	1.7	0.7	83
Prednisolone	6.4	1.5	100	6.7	0.2	100	3.5	1.3	83
Cortisol	1.2	0.9	75	2.7	0.0	75	0.3	0.1	50
Tetrahydrocortisone	< MQL		75				1.4	0.0	83
Corticosterone acetate									
Dexamethasone									
Prednisolone acetate									
Cortisone acetate	< MQL		100						
Hydrocortisone 21-acetate									
2-methyl phenol				2302.1	508.8	100			
4-ethylphenol	1518.0	113.0	100	111.8	22.9	67	58.2	52.0	83
4-isopropyl phenol									
4-chloro-3-methylphenol							28.3	0.0	33
2,5-dichloro phenol									
3,4,6-trichlorophenol									
Bisphenol A							45.8	18.6	67
Dimethyl phthalate									
Diethyl phthalate	336.0	271.0	100	43.0	46.0	100			
Dibutyl phthalate	308.0	297.0	100	791.0	242.0	100	29.1	17.5	83
Diamyl phthalate				12.5	3.0				
Benzyl butyl phthalate				105.0	29.0	100	14.8	18.0	83
Dicyclohexyl phthalate									
Dihexyl phthalate	37.0	9.0	100	23.0	2.0	100			
Dibenzyl phthalate	< MQL		100				6.8	0.1	33
Diethylheyxl phthalate	140.3	103.7	100	517.5	110.7	100	2.1	0.8	67
Dinonyl phthalate							13.1	5.0	67
Diisodecyl phthalate	<MQL		100				8.2	0.5	33
Monomethyl phthalate				1579.7	27.0	67			
Monoethyl phthalate							0.3	0.2	50
Monobutyl phthalate	73.0	28.0	100	109.0	14.0	100			
Mono-n-pentyl phthalate				25.0	42.0	100	1.1	0.2	67
Monocyclohexyl phthalate									
Monohexyl phthalate							10.4	9.3	67
Monobenzyl phthalate							1.9	0.2	50
Monoethylhexyl phthalate				674.4	115.2	100	34.9	37.1	50

Table D10. Overview of the quantitative results obtained in samples from SC2, collected at harbour of Zeebrugge (HZ). During the deployment and retrieval of the passive (P) samplers, active samples were taken; referred to as (D) and (R). Av and SD (n_{active}=3 and $n_{passive}$=6) indicate the average concentration and the standard deviation, respectively, both expressed in ng L^{-1}. DF represents the detection frequency (%). < MQL refers to concentrations below the method quantification limit. Blank cells refer to concentrations below the method detection limit.

	D			R		
	Av	SD	DF	Av	SD	DF
Methandriol						
17α-trenbolone				< MQL		67
17β-trenbolone	0.6	0.1	67	0.7	0.1	100
11β-hydroxyandrosterone				2.0	0.1	67
Testosterone 17β-cypionate						
17β-dihydroandrosterone						
Androsterone	37.3	10.3	67	23.1	3.6	100
19-nortestosterone						
1,4-Androstadienedione				1.2	0.3	100
11-ketoetiocholanolone	2.3	0.6	67	0.9		33
Androstenedione	1.1	0.4	100	0.8	0.3	67
Mestanolone	4.3	0.3	33			
17α-testosterone				0.3	0.2	67
17β-testosterone	0.6		33	< MQL		10
5α-dihydrotestosterone	< MQL		100	< MQL		100
Norethindron				< MQL		100
Methylboldenone	1.9	0.8	67	1.0	0.4	100
11-ketotestosterone	2.3	0.1	67	1.1	0.4	100
Formestane	7.4	3.5	100	3.2	1.4	67
Norethandrolone						
Methyltestosterone						
Trenbolone acetate				0.9	0.1	67
Ethynyl testosterone				0.6	0.0	67
Stanozolol						
Testosterone acetate	4.2	1.7	100	< MQL		100
Fluoxymesterone						
Testosterone propionate	0.7	0.1	33			
Chlorotestosteron acetate						
Testosterone benzoate						
Testosterone phenylpropionate	4.8	4.2	100	2.2	1.7	100
19-nortestosterone-17-decanoate						
17α-estradiol						
17b-estradiol						
Estradiol-17-acetate						
Dienoestrol						
Equilin						
Diethylstilbestrol						
Estrone				0.7	0.2	67
17α-ethinylestradiol						
α-zearalenol						
β-zearalenol	3.2	2.0	67	4.4	0.9	100
α-zeranol	1.8	1.4	67	1.5		33
β-zeranol	3.3	0.5	100	4.1	0.9	67
Gestodene	1.4	0.6	100			
Estradiol-benzoate						
5α-Pregnan-3α,20β-diol						
Norgestrel	0.9	0.1	67	0.7	0.0	67
Dihydroprogesterone						
Progesterone	0.6	0.7	100	< MQL		67
Methylprogesterone	2.4	2.2	100	0.2	0.1	67
17α-hydroxyprogesterone	9.3	9.0	100	1.0	0.2	100
Megestrol						
Medroxyprogesterone	< MQL		100	0.3		100

	D			R		
	Av	**SD**	**DF**	**Av**	**SD**	**DF**
17α-acetoxyprogesterone				0.3		100
Megestrol acetate						
Medroxyprogesterone acetate	2.8	1.0	67	2.9	2.3	67
Flugestone acetate	3.0	0.1	67	2.9	0.4	67
Caproxyprogesterone						
Prednisone	8.2	8.3	67	1.3	0.6	67
Corticosterone						
Cortisone	< MQL		100	< MQL		100
Prednisolone	< MQL		67	3.1	0.2	100
Cortisol	1.7	0.4	67	2.5	0.3	67
Tetrahydrocortisone						
Corticosterone acetate	< MQL		100	< MQL		67
Dexamethasone						
Prednisolone acetate						
Cortisone acetate						
Hydrocortisone 21-acetate	< MQL		100	< MQL		100
2-methyl phenol				157.6		33
4-ethylphenol	1518.0	72.7	100			
4-isopropyl phenol						
4-chloro-3-methylphenol						
2,5-dichloro phenol						
3,4,6-trichlorophenol						
Bisphenol A						
Dimethyl phthalate						
Diethyl phthalate	336.3	370.8	67			
Dibutyl phthalate	307.6	297.3	67			
Diamyl phthalate						
Benzyl butyl phthalate				43.4	13.5	67
Dicyclohexyl phthalate						
Dihexyl phthalate	37.0	8.8	100			
Dibenzyl phthalate						
Diethylheyxl phthalate	184.6	148.2	100	96.4	66.9	67
Dinonyl phthalate						
Diisodecyl phthalate						
Monomethyl phthalate				3165.7	234.4	100
Monoethyl phthalate						
Monobutyl phthalate	58.6	31.4	100	84.2	1.0	67
Mono-n-pentyl phthalate	45.3	6.4	67	77.3	8.9	67
Monocyclohexyl phthalate	70.6	9.5	67			
Monohexyl phthalate				38.5	9.3	67
Monobenzyl phthalate	56.8	3.5	67			
Monoethylhexyl phthalate	399.0	97.6	67	994.5	197.3	67

Table D11. Overview of the quantitative results obtained in samples from SC3, collected at of harbour of Zeebrugge (HZ). During the deployment and retrieval of the passive (P) samplers, active samples were taken; referred to as (D) and (R). Av and SD (n_{active}=3 and $n_{passive}$=6) indicate the average concentration and the standard deviation, respectively, both expressed in ng L^{-1}. DF represents the detection frequency (%). < MQL refers to concentrations below the method quantification limit. Blank cells refer to concentrations below the method detection limit.

	D			R			P		
	Av	SD	DF	Av	SD	DF	Av	SD	DF
Methandriol									
17α-trenbolone	0.7	0.3	67	0.8	0.2	67			
17β-trenbolone	1.0	0.1	67	1.0	0.5	100			
11β-hydroxyandrosterone				0.7	0.1	100			
Testosterone 17β-cypionate									
17β-dihydroandrosterone									
Androsterone	5.6	1.8	67						
19-nortestosterone	5.0	0.9	100						
1,4-Androstadienedione									
11-ketoetiocholanolone							0.1	0.1	67
Androstenedione	1.4	0.3	67						
Mestanolone	14.3	0.8	100						
17α-testosterone	2.5	0.2	67	2.3	0.7	100			
17β-testosterone				0.5		33			
5α-dihydrotestosterone	2.7	2.2	100	2.2	1.8	100			
Norethindron									
Methylboldenone				11.9	14.7	67			
11-ketotestosterone				1.3	0.4	67			
Formestane				10.0	10.7	100			
Norethandrolone	0.4	0.1	67						
Methyltestosterone	0.3	0.2	67						
Trenbolone acetate	0.4	0.3	67						
Ethynyl testosterone	0.7	0.1	67	0.7	0.1	67			
Stanozolol									
Testosterone acetate				1.2	0.1	100			
Fluoxymesterone	< MQL		100						
Testosterone propionate				0.8	0.6	100			
Chlorotestosteron acetate	0.8		33				0.1		17
Testosterone benzoate							0.1	0.0	67
Testosterone phenylpropionate				4.1	0.7	100	0.3	0.2	67
19-nortestosterone-17-decanoate									
17α-estradiol	< MQL		33						
17β-estradiol	19.0	13.3	100	6.8	0.9	67			
Estradiol-17-acetate	11.1	2.7	100	4.5	2.7	100			
Dienoestrol				< MQL		67			
Equilin				0.9		33	2.3	0.9	67
Diethylstilbestrol									
Estrone				< MQL		67			
17α-ethinylestradiol	< MQL		67	< MQL		67	2.2	1.3	50
α-zearalenol									
β-zearalenol				5.3	1.2	100			
α-zeranol	< MQL		100	< MQL		100			
β-zeranol	< MQL		100						
Gestodene									
Estradiol-benzoate							1.3	1.5	33
5α-Pregnan-3α,20β-diol									
Norgestrel				0.6	0.1	67			
Dihydroprogesterone	1.1	0.1	67						
Progesterone	13.4	1.1	100	7.4	1.2	100	0.2	0.0	50
Methylprogesterone	2.0	0.1	67				0.2	0.0	50
17α-hydroxyprogesterone	8.0	2.3	67	8.0	1.3	100	1.0	0.3	50
Megestrol				1.4		33			
Medroxyprogesterone	0.6	0.0	67	0.7	0.2	67			

	D			R			P		
	Av	SD	DF	Av	SD	DF	Av	SD	DF
17α-acetoxyprogesterone				1.8	0.0	67			
Megestrol acetate				< MQL		67			
Medroxyprogesterone acetate	1.5	1.4	100	< MQL		67			
Flugestone acetate	1.3		33	1.6	0.1	67			
Caproxyprogesterone	1.2	0.0	100	1.6		33	0.3		17
Prednisone				27.9	7.3	100	1.8	0.4	33
Corticosterone	4.4	0.4	100	4.1	0.4	67			
Cortisone				6.6	1.7	67	0.4	0.3	33
Prednisolone	18.5	0.3	67	6.5	0.1	67	1.4	0.1	50
Cortisol							0.2	0.1	50
Tetrahydrocortisone	4.7	3.1	67	< MQL		33			
Corticosterone acetate				< MQL		67			
Dexamethasone	9.7	0.1	67	10.9	0.1	67	2.3	0.0	50
Prednisolone acetate									
Cortisone acetate							20.1	2.8	50
Hydrocortisone 21-acetate									
2-methyl phenol	173.0	53.4	67						
4-ethylphenol	2674.0	394.4	67	388.5	254.1	100	7.2	1.2	83
4-isopropyl phenol							82.4	1.6	83
4-chloro-3-methylphenol									
2,5-dichloro phenol							14.5	0.2	100
3,4,6-trichlorophenol							9.1	0.1	50
Bisphenol A									
Dimethyl phthalate							10.0		17
Diethyl phthalate	< MQL		100						
Dibutyl phthalate	668.2	146.5	67	322.2	171.1	100	37.6	4.6	100
Diamyl phthalate	< MQL		100				138.8	20.5	100
Benzyl butyl phthalate				95.1	55.8	100	4.4	0.2	50
Dicyclohexyl phthalate				58.8	5.3	67	18.3	5.1	100
Dihexyl phthalate							94.8	36.4	100
Dibenzyl phthalate				22.3		33	6.8	0.1	50
Diethylheyxl phthalate	399.7	239.4	67	248.6	316.4	100	2.3	1.2	100
Dinonyl phthalate				271.1	229.4	67	63.1	31.3	100
Diisodecyl phthalate							12.1	2.0	67
Monomethyl phthalate							23.9	11.5	83
Monoethyl phthalate							1.1	0.3	50
Monobutyl phthalate	28.4	3.5	67						
Mono-n-pentyl phthalate							6.3	6.3	100
Monocyclohexyl phthalate							0.9	0.5	83
Monohexyl phthalate							22.8	8.9	100
Monobenzyl phthalate							3.2	1.3	83
Monoethylhexyl phthalate							14.1	3.7	67

Table D12. Overview of the quantitative results obtained in samples from SC4, collected at harbour of Zeebrugge (HZ). During the deployment and retrieval of the passive (P) samplers, active samples were taken; referred to as (D) and (R). Av and SD (n_{active}=3 and $n_{passive}$=6) indicate the average concentration and the standard deviation, respectively, both expressed in ng L^{-1}. DF represents the detection frequency (%). < MQL refers to concentrations below the method quantification limit. Blank cells refer to concentrations below the method detection limit.

	D			R			P		
	Av	SD	DF	Av	SD	DF	Av	SD	DF
Methandriol							29.3	13.0	100
17α-trenbolone									
17β-trenbolone	< MQL		100						
11β-hydroxyandrosterone									
Testosterone 17β-cypionate									
17β-dihydroandrosterone									
Androsterone									
19-nortestosterone				0.9	0.1	100			
1,4-Androstadienedione									
11-ketoetiocholanolone				0.3	0.1	100			
Androstenedione									
Mestanolone									
17α-testosterone									
17β-testosterone	0.3		33						
5α-dihydrotestosterone	< MQL		100	< MQL		100	14.6	1.2	100
Norethindron									
Methylboldenone	0.7	0.4	100						
11-ketotestosterone									
Formestane									
Norethandrolone	0.3	0.0	67						
Methyltestosterone	0.4	0.1	100	0.4	0.0	100			
Trenbolone acetate	< MQL		67						
Ethynyl testosterone	0.8		33	< MQL		100			
Stanozolol									
Testosterone acetate				< MQL		100			
Fluoxymesterone									
Testosterone propionate	0.3	0.0	100						
Chlorotestosteron acetate									
Testosterone benzoate							0.1	0.0	100
Testosterone phenylpropionate							0.1	0.0	33
19-nortestosterone-17-									
17α-estradiol									
17β-estradiol	< MQL		67				35.1	3.1	100
Estradiol-17-acetate									
Dienoestrol									
Equilin							3.5	0.9	67
Diethylstilbestrol				0.3		33			
Estrone				0.6	0.5	67			
17α-ethinylestradiol									
α-zearalenol									
β-zearalenol				0.5	0.2	100			
α-zeranol									
β-zeranol				< MQL		67			
Gestodene	1.0	0.0	67	< MQL		67			
Estradiol-benzoate							1.3	1.1	50
5α-Pregnan-3α,20β-diol									
Norgestrel	2.8	1.0	67						
Dihydroprogesterone				0.4		33			
Progesterone							0.2	0.1	100
Methylprogesterone									
17α-hydroxyprogesterone	0.3	0.0	100						
Megestrol									
Medroxyprogesterone	< MQL		33						

	D			R			P		
	Av	SD	DF	Av	SD	DF	Av	SD	DF
17α-acetoxyprogesterone	0.8	0.8	67						
Megestrol acetate									
Medroxyprogesterone acetate									
Flugestone acetate									
Caproxyprogesterone									
Prednisone							2.3	0.7	100
Corticosterone									
Cortisone	< MQL		67				2.2	1.7	100
Prednisolone				< MQL		67	3.2	1.8	100
Cortisol							0.9	0.7	100
Tetrahydrocortisone									
Corticosterone acetate	141.7		100						
Dexamethasone							2.8	0.4	100
Prednisolone acetate									
Cortisone acetate							254.9	87.3	100
Hydrocortisone 21-acetate									
2-methyl phenol	363.5	43.9	67						
4-ethylphenol	2204.3	2730.1	100	203.9	28.8	100			
4-isopropyl phenol	354.6	333.6	100						
4-chloro-3-methylphenol									
2,5-dichloro phenol									
3,4,6-trichlorophenol				74.4	6.0	67			
Bisphenol A	273.2	180.6	100	194.9	32.2	67			
Dimethyl phthalate									
Diethyl phthalate									
Dibutyl phthalate	296.3	218.9	100	34.1	6.6	100			
Diamyl phthalate	< MQL		67						
Benzyl butyl phthalate	32.2	22.3	100	41.8	5.3	100			
Dicyclohexyl phthalate				51.0	4.1	100			
Dihexyl phthalate	< MQL		100						
Dibenzyl phthalate									
Diethylheyxl phthalate	604.7	353.4	100	109.7	62.2	100			
Dinonyl phthalate	90.4	63.1	67						
Diisodecyl phthalate	107.9	5.3	67	77.2	0.3	100	7.9	0.0	33
Monomethyl phthalate									
Monoethyl phthalate				767.2	223.0	100			
Monobutyl phthalate									
Mono-n-pentyl phthalate	759.4	1129.9	100	183.2	73.5	100			
Monocyclohexyl phthalate				271.6	174.8	100			
Monohexyl phthalate	538.4	360.9	100	256.3	150.8	100			
Monobenzyl phthalate				114.2	30.0	100			
Monoethylhexyl phthalate	2654.9	3514.8	100	214.4	122.7	100			

Table D13. Overview of the quantitative results obtained in samples from SC2, collected at open sea of Oostende (OO). During the deployment and retrieval of the passive (P) samplers, active samples were taken; referred to as (D) and (R). Av and SD indicate the average concentration and the standard deviation, respectively, both expressed in ng L^{-1}. DF (n_{active}=3 and $n_{passive}$=6) represents the detection frequency (%). < MQL refers to concentrations below the method quantification limit. Blank cells refer to concentrations below the method detection limit.

	D			R			P		
	Av	SD	DF	Av	SD	DF	Av	SD	DF
Methandriol							58.0	28.2	100
17α-trenbolone	2.3	1.2	67	1.3	1.4	67			
17β-trenbolone	1.0	0.0	67	1.3	0.1	100			
11β-hydroxyandrosterone	1.8	0.9	67	1.4	0.2	67			
Testosterone 17β-cypionate									
17β-dihydroandrosterone									
Androsterone	45.7	31.1	100	168.8	30.5	100			
19-nortestosterone	6.4	0.9	67						
1,4-Androstadienedione	1.0	0.0	67	1.2	0.3	100			
11-ketoetiocholanolone	1.3	0.3	67	1.3	0.2	67	2.0	1.0	100
Androstenedione	0.8	0.0	67	1.7	0.1	67			
Mestanolone	127.5	179.1	67	3.3	0.3	67			
17α-testosterone	0.7	0.1	100	2.4	0.2	67			
17β-testosterone	< MQL		100	0.3	0.1	67			
5α-dihydrotestosterone	< MQL		100	< MQL		100			
Norethindron	< MQL		100	1.5	0.5	67			
Methylboldenone	2.3	0.2	100	1.5	0.4	67			
11-ketotestosterone	1.5	0.2	100	1.4	0.2	100			
Formestane	3.6	1.1	67	3.0	0.7	100			
Norethandrolone	1.4	0.1	67	2.1	0.9	100			
Methyltestosterone				1.8	0.3	67			
Trenbolone acetate	1.8	0.1	67	2.9	0.2	67			
Ethynyl testosterone				4.3	0.3	100			
Stanozolol									
Testosterone acetate	0.6	0.3	67	3.2	1.7	100			
Fluoxymesterone									
Testosterone propionate									
Chlorotestosteron acetate				0.7		33			
Testosterone benzoate									
Testosterone phenylpropionate	2.2	2.6	67	15.0	20.5	100	1.3	1.4	100
19-nortestosterone-17-decanoate									
17α-estradiol									
17β-estradiol							47.8	14.2	100
Estradiol-17-acetate									
Dienoestrol									
Equilin				7.7	2.5	33	70.3	5.9	50
Diethylstilbestrol				14.4		33			
Estrone	1.5		33	0.8		33			
17α-ethinylestradiol									
α-zearalenol	3.2	1.3	67	5.1	1.5	100			
β-zearalenol	2.4	1.8	67	5.9	1.8	67			
α-zeranol	1.5	0.3	100	1.9	1.2	100			
β-zeranol	4.0	0.7	67	4.2	1.1	67			
Gestodene	1.3	0.1	67	1.6	0.6	67			
Estradiol-benzoate									
5α-Pregnan-3α,20β-diol									
Norgestrel				1.9	0.4	100			
Dihydroprogesterone									
Progesterone	< MQL		67	0.3	0.0	100	0.5	0.2	100
Methylprogesterone				0.8	0.3	67			
17α-hydroxyprogesterone	50.4	86.3	100	3.2	0.7	67			
Megestrol									
Medroxyprogesterone	< MQL		67	0.5	0.3	67			

	D			R			P		
	Av	SD	DF	Av	SD	DF	Av	SD	DF
17α-acetoxyprogesterone	5.2	0.5	67				1.4	0.8	100
Megestrol acetate	11.7	2.3	67						
Medroxyprogesterone acetate	141.5	10.4	67						
Flugestone acetate	2.8	0.3	67	2.8	0.2	100	1.0	0.6	100
Caproxyprogesterone	132.0	23.5	67						
Prednisone	1.0	0.4	67	2.3	0.2	67	4.8	3.0	100
Corticosterone	< MQL		33						
Cortisone	103.5	144.6	67	< MQL		100	21.7	14.2	100
Prednisolone	< MQL		33	2.6	1.3	100	18.4	10.0	100
Cortisol	2.3		33	2.3	1.0	100	8.0	4.7	100
Tetrahydrocortisone									
Corticosterone acetate	< MQL		67	< MQL		67			
Dexamethasone	68.7		33	6.6		33			
Prednisolone acetate	25.4		33						
Cortisone acetate							50.5	18.6	100
Hydrocortisone 21-acetate	< MQL		67	< MQL		67	40.6		33
2-methyl phenol	< MQL		100	203.2		33			
4-ethylphenol	275.0	198.9	100	593.9	345.1	67	37.4	22.2	100
4-isopropyl phenol									
4-chloro-3-methylphenol									
2,5-dichloro phenol									
3,4,6-trichlorophenol									
Bisphenol A							45.1	30.7	100
Dimethyl phthalate									
Diethyl phthalate				110.2	14.5	67			
Dibutyl phthalate	341.7	274.3	100	157.2	85.6	100	30.1	16.0	100
Diamyl phthalate									
Benzyl butyl phthalate				99.3	66.9	100	8.8	8.0	100
Dicyclohexyl phthalate				41.6	18.4	33			
Dihexyl phthalate				26.2	7.8	67			
Dibenzyl phthalate									
Diethylheyxl phthalate	122.4	59.0	100	168.9	94.0	100	2.3	1.8	83
Dinonyl phthalate							14.5	11.2	83
Diisodecyl phthalate									
Monomethyl phthalate	1094.9	325.4	67	6103.5	649.6	67			
Monoethyl phthalate									
Monobutyl phthalate	45.5	24.5	100	228.9	111.8	100			
Mono-n-pentyl phthalate	69.7	23.4	67	103.4	58.1	100			
Monocyclohexyl phthalate									
Monohexyl phthalate									
Monobenzyl phthalate	57.4	38.3	67	58.0	14.4	67			
Monoethylhexyl phthalate				423.3	52.6	100	40.2	11.7	83

Table D14. Overview of the quantitative results obtained in samples from SC3 and 4, collected at open sea of Oostende (OO). During the deployment and retrieval of the passive (P) samplers, active samples were taken; referred to as (D) and (R). Av and SD (n_{active}=3 and $n_{passive}$=6) indicate the average concentration and the standard deviation, respectively, both expressed in ng L^{-1}. DF represents the detection frequency (%). < MQL refers to concentrations below the method quantification limit. Blank cells refer to concentrations below the method detection limit.

	SC3						SC4		
	D			R			D		
	Av	SD	DF	Av	SD	DF	Av	SD	DF
Methandriol									
17α-trenbolone	0.5	0.2	67						
17β-trenbolone	0.4	0.3	67				< MQL		100
11β-hydroxyandrosterone	0.8	0.2	67	< MQL		100			
Testosterone 17β-cypionate	1.7	0.8	67						
17β-dihydroandrosterone	3.9		33						
Androsterone				2.1	1.4	67			
19-nortestosterone	2.7	1.6	100						
1,4-Androstadienedione	1.9	0.1	67	1.0	0.0	67			
11-ketoetiocholanolone							< MQL		100
Androstenedione									
Mestanolone									
17α-testosterone	2.4	0.1	67						
17β-testosterone							0.3	0.0	100
5α-dihydrotestosterone	< MQL		100	0.4	0.0	100	0.2	0.1	100
Norethindron	3.2	0.7	67						
Methylboldenone	1.3	1.2	67				< MQL		100
11-ketotestosterone	1.0	0.2	67	1.4	0.1	67	0.4	0.1	100
Formestane	1.5	0.7	67						
Norethandrolone							0.2	0.1	67
Methyltestosterone	0.4	0.2	100	< MQL		67	0.4	0.0	67
Trenbolone acetate							< MQL		100
Ethynyl testosterone	0.6	0.1	67				0.3	0.0	100
Stanozolol									
Testosterone acetate				0.9	0.0	100			
Fluoxymesterone									
Testosterone propionate				1.2	0.1	100	0.3	0.0	100
Chlorotestosteron acetate	0.6		33						
Testosterone benzoate	1.3	0.1	67						
Testosterone phenylpropionate	3.6	4.5	100						
19-nortestosterone-17-decanoate									
17α-estradiol	< MQL		100						
17β-estradiol	7.6	2.5	100	7.4	0.4	100			
Estradiol-17-acetate	1.5	0.5	100	2.2	0.2	100			
Dienoestrol									
Equilin									
Diethylstilbestrol									
Estrone									
17α-ethinylestradiol									
α-zearalenol							< MQL		100
β-zearalenol							1.3	0.9	67
α-zeranol	< MQL		67						
β-zeranol	< MQL		100						
Gestodene									
Estradiol-benzoate	< MQL		100						
5α-Pregnan-3α,20β-diol									
Norgestrel	0.4	0.0	67				2.6	0.1	67
Dihydroprogesterone									
Progesterone	8.2	5.0	100						
Methylprogesterone	1.2	0.1	100						
17α-hydroxyprogesterone				0.8	0.1	100	0.3	0.0	100
Megestrol	1.3	0.0	67						

	SC3 D			R			SC4 D		
	Av	SD	DF	Av	SD	DF	Av	SD	DF
Medroxyprogesterone	0.4	0.2	67	0.3	0.1	100			
17α-acetoxyprogesterone	1.8	0.0	100				< MQL		67
Megestrol acetate	0.8	0.4	100	< MQL		100			
Medroxyprogesterone acetate	3.6	3.3	100						
Flugestone acetate	1.2	0.4	67						
Caproxyprogesterone	1.3	0.2	67						
Prednisone									
Corticosterone	3.6	1.7	67	2.3	1.5	67			
Cortisone	2.8	0.3	67	5.7	0.6	67			
Prednisolone	8.2	3.9	67	15.2	3.0	67			
Cortisol									
Tetrahydrocortisone	< MQL		67	< MQL		100			
Corticosterone acetate							279.2	365.2	67
Dexamethasone	9.5		33						
Prednisolone acetate									
Cortisone acetate									
Hydrocortisone 21-acetate									
2-methyl phenol	326.4		33	429.9		33			
4-ethylphenol	823.0	761.6	67	217.1	105.5	100	2628.7	303.4	67
4-isopropyl phenol				329.2	143.4	100	310.4	193.9	33
4-chloro-3-methylphenol									
2,5-dichloro phenol				532.8	497.1	100			
3,4,6-trichlorophenol				32.0		33			
Bisphenol A				1413.1	261.4	100	429.0	75.8	67
Dimethyl phthalate									
Diethyl phthalate				1619.7	1363.6	100			
Dibutyl phthalate	1743.4	234.7	100	1443.6	626.6	100	61.7		33
Diamyl phthalate				80.3		33			
Benzyl butyl phthalate				285.8	94.8	100	47.4	8.9	67
Dicyclohexyl phthalate	< MQL		100	216.3	82.0	100			
Dihexyl phthalate									
Dibenzyl phthalate				65.4	16.9	67			
Diethylheyxl phthalate	37.0	12.4	67	119.0	66.6	100	357.6		33
Dinonyl phthalate				52.7	38.4	67	395.2	68.4	67
Diisodecyl phthalate							115.1		33
Monomethyl phthalate				34.5	8.4	33			
Monoethyl phthalate									
Monobutyl phthalate	< MQL		67	1549.2	1302.5	100			
Mono-n-pentyl phthalate				< MQL		100	185.8		33
Monocyclohexyl phthalate	54.5	28.4	67	35.8	19.9	100			
Monohexyl phthalate							668.3		33
Monobenzyl phthalate				40.8	10.0	100			
Monoethylhexyl phthalate							1093.1	208.4	67

Summary

Summary

Nowadays, a growing societal and scientific concern exists regarding the widespread occurrence of endocrine disrupting compounds (EDCs) in the aquatic environment. The most important EDC classes comprise the steroid hormones, plasticizers and plastics additives. Indeed, even at very low concentrations, steroidal EDCs may affect the hormonal system of aquatic organisms, while leaching of plasticizers and plastics additives into the aquatic environment is inevitable as a result of their extensive use for numerous applications. Until now, limited knowledge was available on the presence of steroidal EDCs, plasticizers and plastics additives in the marine environment. Therefore, an urgent need existed to monitor the fate and effects, as well as the environmental and human risks posed by these emerging micropollutants in marine ecosystems. Given our lack of knowledge on the occurrence of EDCs, the **main goal of this dissertation** was to investigate the prevalence of steroidal EDCs, plasticizers and plastics additives in the marine environment. To this end, new analytical methods targeting a broad range of EDCs (n=97) were developed, optimized and validated. Both active, as well as passive sampling approaches were applied extensively in the Belgian Part of the North Sea (BPNS), from 2016 to 2018. Passive sampling involves a collecting device, placed *in situ,* which accumulates chemical pollutants in the environment over time, while for active sampling, a sample is taken directly from the environment at one point in time. Finally, the potential negative effects of the detected compound concentrations were assessed.

Chapter I entails a comprehensive overview on the fate of EDCs in the aquatic environment. Additionally, a summary on the predominant analytical strategies employed for sampling, extraction and detection of EDCs is provided, including a detailed description of the analytical platforms and their capabilities. This chapter ends with the conceptual framework and research objectives of this PhD study, as outlined in research chapters II, III and IV.

The aim of **chapter II** was to develop and validate two accurate ultra-high performance liquid chromatography (UHPLC) coupled to high-resolution mass spectrometry (HRMS) methods for

the quantification of steroidal EDCs (n=70), plastics additives and plasticizers (n=27) in seawater. For each target group, the instrumental methods were combined with optimised large-volume solid phase extraction procedures, as it was the goal to detect ng.L^{-1} range residues in the marine environment. The developed methods showed excellent performance characteristics (according to 2002/657/EC and Eurachem guidelines) and versatility to fresh water samples.

In **chapter III**, a new sorbent phase for passive sampling purposes was investigated that enables to capture a broad polarity range of emerging organic compounds (log P range between 1.30 to 9.85). For 131 compounds the sampler-water partition coefficients (K_{sw}) could be determined by using a static exposure design. Calculation of the thermodynamic parameters indicated that the main partitioning process for the (alkyl)phenols, personal care products, pesticides and pharmaceuticals was driven by physisorption, while the uptake of phthalates and steroidal EDCs was mediated by a combination of physisorption and chemisorption. It was clearly demonstrated in this chapter that hydrophilic DVB can be used for passive sampling.

Using the newly developed methodologies from chapters II and III, active and passive samplers were intensively deployed in the Belgian Part of the North Sea, as discussed in **chapter IV**. The detected concentrations for the steroidal EDCs were mainly below 10 ng L^{-1}, while for the plastics additives and plasticizers concentrations between 10 and 1000 ng L^{-1} were observed. Concentrations detected by passive sampling, were generally lower than those in active samples. Furthermore, it could be concluded that our proposed strategy for active and passive sampling offers a complementary approach for the measurement of EDCs in the marine environment. Ultimately, the detected concentrations of 17β-estradiol exceeded pose a risk, as the EQS of 0.08 ng L^{-1} was exceeded. No risk was observed for diethylhexyl phthalate, although this was the most abundant plasticizer.

Finally, in **chapter V**, the general discussion and future perspectives of this PhD study are presented. Thereby, it was suggested that future research should focus on the implementation and use of an atomized analysis pipeline for suspect and untargeted screening. Also, the

development of new passive samplers containing hydrophilic DVB could be promising, as Speedisks showed reduced K_{sw} as compared to naked sorbent. Moreover, Speedisks could serve as a viable archive tool for sampled EDCs, but this should be investigated more extensively. Finally, a more in-depth evaluation of contaminant levels in Speedisk passive sampler as compared to those in biota could offer an added value towards environmental risk assessment of the findings obtained in this PhD.

Overall, this thesis contributes to the establishment of integrated active and passive sampling approaches for monitoring EDCs in the marine environment, and more specifically the BPNS.

Samenvatting

Samenvatting

Momenteel heerst vanuit maatschappelijk en wetenschappelijk oogpunt een toenemende bezorgdheid omtrent de aanwezigheid van hormoonverstorende stoffen (EDC's) in het aquatisch milieu. Tot de meest belangrijke EDC-klassen behoren de steroïdhormonen, de weekmakers en de kunststofadditieven aangezien steroïdhormonen reeds aan zeer lage concentraties het hormonaal systeem van waterorganismen kunnen verstoren en het voorkomen van weekmakers en kunststofadditieven onvermijdelijk is door hun veelvuldig gebruik in talrijke toepassingen. Tot op heden bestond weinig kennis omtrent de aanwezigheid van steroïdhormonen, weekmakers en kunststofadditieven in het mariene milieu. Het was aldus noodzakelijk om de bestemming en effecten, alsook de risico's van EDC's voor zowel milieu als mens te bestuderen in het mariene ecosysteem. Gezien de beperkte kennis omtrent de aanwezigheid van EDCs, bestond het **hoofddoel van dit doctoraat** erin om de prevalentie van steroïdhormonen, weekmakers en kunststofadditieven in het mariene milieu te evalueren. Hiervoor werden nieuwe methodes voor een breed bereik aan EDC's (n=97) ontwikkeld, geoptimaliseerd en gevalideerd. Bovendien werden zowel actieve als passieve bemonsteringstechnieken bestudeerd en intensief toegepast in het Belgisch deel van de Noordzee gedurende een periode van 2 jaar (2016-2018). Bij passieve bemonstering wordt een sampler voor een specifieke periode in situ geplaatst, gedurende dewelke chemische stoffen accumuleren, terwijl bij actieve bemonstering waterstalen rechtstreeks uit de omgeving worden verzameld. Tenslotte werden de potentiële negatieve effecten van de geobserveerde EDC-concentraties en de bijhorende ecotoxicologische implicaties geëvalueerd.

Hoofdstuk I start met een overzicht van het lot/voorkomen van EDC's in het aquatisch milieu. Daarnaast biedt dit hoofdstuk een samenvatting van de overheersende analytische strategieën voor bemonstering, met onder meer een gedetailleerde beschrijving van het analytisch instrumentarium en zijn mogelijkheden. Ten slotte wordt het conceptueel kader geschetst en de opbouw van de verschillende onderzoeksfasen aangegeven, zoals voorgesteld in hoofdstukken II, III en IV.

Hoofdstuk II beschrijft de ontwikkeling en validatie van twee ultrahoge performantie vloeistofchromatografie (UHPLC) gekoppeld aan hoge resolutie massaspectrometrie (HRMS)-gebaseerde methodes voor de kwantificatie van steroïdhormonen (n=70), weekmakers en kunststofadditieven (n=27) in zeewater. Voor iedere EDC-klasse werd de instrumentele methode gecombineerd met een geoptimaliseerde groot-volume vaste-fase extractie, aangezien deze methodes finaal zouden worden aangewend voor de detectie van residuen aan ng.L^{-1} concentraties in het mariene milieu. Beide methodes werden succesvol gevalideerd (volgens CD 2002/657/EC en Eurachem richtlijnen) en toonden eveneens potentieel voor het analyseren van zoetwaterstalen.

In **hoofdstuk III** werd een nieuw sorptiemiddel onderzocht voor de passieve bemonstering van een groot aantal organische contaminanten met uiteenlopende polariteit (log P varieert tussen 1.30 en 9.85). Voor 131 componenten werd de sorbent/water-partitiecoëfficiënt (K_{sw}) bepaald door middel van een statisch blootstellingsmodel. Na het berekenen van de thermodynamische paramaters kon worden afgeleid dat het sorptieproces voor de (alkyl)fenolen, persoonlijke verzorgingsproducten, pesticiden en farmaceutica voornamelijk werd gedreven door fysisorptie, terwijl de opname van ftalaten en steroïdhormonen bestond uit een combinatie van zowel fysisorptie als chemisorptie. Dit hoofdstuk toonde duidelijk aan dat hydrofiel DVB kan worden gebruikt als sorbent voor passieve bemonstering.

Op basis van de nieuwe ontwikkelingen in hoofdstuk II en III, werden actieve en passieve bemonsteringsmethodes intensief toegepast in het Belgisch deel van de Noordzee (**hoofdstuk IV**). De gedetecteerde concentraties van de steroïdhormonen bleven hoofdzakelijk onder 10 ng L^{-1}, terwijl voor de weekmakers en kunststofadditieven concentraties tussen 10 en 1000 ng L^{-1} werden geobserveerd. In het algemeen waren de concentraties na passieve bemonstering lager dan deze door actieve bemonstering. Daarenboven kon worden vastgesteld dat de vooropgestelde strategie van actieve en passieve bemonstering complementair was voor de bepaling van EDC's in het mariene milieu. Tenslotte werd met behulp van een risico-analyse aangetoond dat 17β-estradiol een risico vormt, aangezien de omgeving kwaliteit standaard van 0.08 ng L^{-1} werd overschreden. Geen

risico werd waargenomen voor diethylhexyl ftalaat, desondanks dit de meest voorkomende gedetecteerde weekmaker was.

Ter afsluiting werden naast de algemene discussie ook de toekomstperspectieven van dit doctoraal onderzoek beschreven in **hoofdstuk V**. Hierbij werd geopperd dat toekomstig onderzoek zich zou moeten richten op de implementatie en het gebruik van een automatische analysepipeline voor verdachte en niet-gerichte screening. Daarnaast zou de ontwikkeling van een nieuwe passieve bemonsteringstechniek veelbelovend kunnen zijn, aangezien de Speedisk een lagere K_{sw} vertoonde dan het naakte sorbent. Vervolgens moet verder worden nagegaan of de lange-termijn bewaring van EDC's op Speedisks een mogelijke piste kan bieden. Tenslotte kan een meer diepgaande vergelijking van verontreiniging in Speedisks en biota een meerwaarde bieden in omgevingsgebonden risico-analyse, zoals beschreven in dit doctoraat.

Over het algemeen kan worden geconcludeerd dat dit doctoraal onderzoek heeft bijgedragen aan het verwerven van essentiële informatie omtrent geïntegreerde actieve en passieve bemonsteringstechnieken voor de bepaling van EDC's in het mariene milieu en in het bijzonder het Belgisch deel van de Noordzee.

Curriculum vitae

PERSONALIA

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Date of birth:	1992-03-05
Place of birth:	Bruges
Nationality:	Belgian

EDUCATION

PhD research (10/2015 – 9/2019)

- Ghent University, Faculty of Veterinary Medicine, Department of Veterinary Public Health and Food Safety, Laboratory of Chemical Analysis

Master of Science in Engineering Technology in Chemical Sciences, Ghent University (6/2011 – 6/2015)

- "Chemical-speciation, removal and recuperation of heavy metals from wastewater"
- Ghent University, Faculty of Bioscience Engineering, Laboratory of Water- and Ecotechnology
- Ghent University, Faculty of Bioscience Engineering, Laboratory of Analytical Chemistry and Applied Ecochemistry

TRAINING AND CERTIFICATES

Doctoral schools (10/2015 – 9/2019)

Transferable skills

- Introduction Day for new PhD students (4th of February, 2016, Ghent Belgium)
- Writing abstracts (24th of February, 2016, Ghent, Belgium)
- Advanced Academic English: Conference Skills – Academic Posters (14th & 21th of March, 2016, Ghent, Belgium)
- Leadership Foundation Course (5th, 6th & 7th of December, 2016, Ghent, Belgium)
- Advanced Academic English: Writing Skills (13th February till 22nd May, 2017, Ghent, Belgium)
- Research integrity in life sciences research – training (6th of December, 2017, Ghent, Belgium)

Specialist courses

- Start using R "for health scientist" (28th of November and 1th, 5th, 8th, 12th & 15th of December, 2017, Ghent, Belgium)
- Data manipulation, analysis and visualization in python (18th, 19th & 20th of December, 2017, Ghent, Belgium)
- International Short course on Advanced High-resolution 3D imaging (17th, 18th & 19th of January, 2018, Ghent, Belgium)

Additional courses and workshops

- Data scientist's Toolbox – John Hopkins University (2015) (Baltimore (US), 2015)
- Clinical Seminar (16th of November, 2015, Brussels, Belgium)
- Vliz Marine Scientist Day (12th of February, 2016, Bruges, Belgium)
- 8th International passive sampling workshop and symposium (7th, 8th, 9th & 10th of September, 2016, Prague, Czech Republic)
- Trace contaminant screening workshop (15th & 16th of November, 2016, Manchester, United Kingdom)
- Marine Data Archive explanations (22nd of November, 2017, Ghent, Belgium)
- New Developments in Quantitation of Small Molecules using LC-MS/MS (12th of October, 2017, Brussels, Belgium)
- 23rd National Symposium & Workshop for Applied Biological Sciences (8th of February, 2018, Brussels, Belgium)
- Vliz Marine Scientist Day (21th of March, 2018, Bredene, Belgium)
- Career day @ Janssen Pharmaceutica (20th of April, 2018, Beerse, Belgium)
- Optimizing Results With Data-Driven experimental Design (24th of April, 2018, Eindhoven, Netherlands)
- 10th International Passive Sampling Workshop and Symposium (9th, 10th & 11th of May, 2018, Dublin, Northern-Ireland)

Different courses succeeded at Flames Statistics (2017 – 2018)

- Comparing samples, non-parametric tests, analysis of variance and blocking and multivariate statistics

Accreditation program (2015)

- Good laboratory practises and analysis (in compliance with the ISO 17025)

PROFESSIONAL ACTIVITIES

Scientific Researcher, Queen's University, United Kingdom (5/2018 – 6/2018)

- Characterization of materials using advanced analytical techniques, i.e. FTIR, NIR and RAMAN.

Assisting practical teaching sessions, Ghent University (2016 – 2018)

- 'Veterinary public health III: food safety and control' for the session 'Chemical Hazards' (in 2nd Master of Veterinary Medicine, 2016-2017)
- 'Veterinary public health IV: food safety and control' for the session 'Chemical Hazards' (in 2nd Master of Veterinary Medicine, 2017-2018)
- 'Veterinary public health: food safety and auditing' for the session 'Chemical Hazards' (in 3nd Master of Veterinary Medicine, 2017-2018)

STUDENTS

Tutor of different dissertations

- Laura Vandenbussche (Bachelor in Chemistry, 2017 – 2018): "Analysis of endocrine disrupting substances in seawater using UHPLC-HR-Q-Orbitrap™-MS"
- Dieter Deprez (Master of Science in Engineering Technology in Biochemical Sciences, 2017– 2018):" Kinetic study of hydrophilic divinylbenzene Speedisks™ as a useful tool for monitoring phthalates and alkylphenols in the marine environment"
- Kelly De groen (Master of Veterinary Medicine, 2016 – 2018): "Antibiotics in cattle breeding and the corresponding impact on the environment"
- Celine Callewaert, Bram Lafroot, Liselot Tack and Jolien Van Dyck (Bachelor of Science in Bio-science, 2016 – 2017): "Emerging organic micro-pollutants in the marine environment: What can we learn from passive sampling?"
- Celeste Verbeke (Master of Science in Engineering Technology in Biochemical Sciences, 2016 - 2017): "Development of SPE-UHPLC-MS/MS method for the simultaneous determination of antibiotics in seawater"

Member of the reading and examination committee

- Bruno D'Argent (Master of Science in Engineering Technology in Biochemical Sciences, 2016 - 2017): "Development of a SPE-GC/MS method for the analysis of carbamazepine and diclofenac in water"
- Celeste Verbeke (Master of Science in Engineering Technology in Biochemical Sciences, 2016 - 2017): "Development of SPE-UHPLC-MS/MS method for the simultaneous determination of antibiotics in seawater"

PUBLICATIONS

Journal articles (A1)

- **Huysman S**, Vanryckeghem F, De Paepe E, Smedes F, A. Haughey S, T. Elliott C, Demeestere K, Vanhaecke L. "Hydrophilic divinylbenzene for equilibrium sorption of emerging organic micropollutants in aquatic matrices" *submitted to Environmental Science & Technology*
- De Paepe E, Wauters J, Van Der Borght M, Claes J, **Huysman S**, Croubels S, Vanhaecke L. "Ultra-High-Performance Liquid Chromatography coupled to Quadrupole Orbitrap High-Resolution Mass Spectrometry for Multi-Residue Screening of Pesticides, (Veterinary) Drugs and Mycotoxins in Edible Insects." FOOD CHEMISTRY (2019)
  Impact factor: 4.946, category: FOOD SCIENCE & TECHNOLOGY, rank: 7/133.
- Moeris S, Vanryckeghem F, **Huysman S**, Vanhaecke L, Demeestere K, De Schamphelarere K. "Combining biotesting and multivariate statistics to explain growth stimulation effects of environmentally realistic contaminant mixtures." ENVIRONMENTAL TOXICOLOGY AND CHEMISTRY (2019)
  Impact factor: 3.179, category: ENVIRONMENTAL SCIENCES, rank: 68/242.
- Vanryckeghem F, **Huysman S**, Van Langenhove H, Vanhaecke L, Demeestere K, " Multi-residue quantification and screening of emerging organic micropollutants in the Belgian Part of the North Sea by use of Speedisk extraction and Q-Orbitrap HRMS." MARINE POLLUTION BULLETIN (2019)
  Impact factor: 3.241, category: MARINE & FRESHWATER BIOLOGY, rank: 9/106.
- **Huysman S**, Van Meulebroek L, Janssens O, Vanryckeghem F, Van Langenhove H, Demeestere K, Vanhaecke L. "Targeted Quantification and Untargeted Screening of Alkylphenols, Bisphenol A and Phthalates in Aquatic Matrices Using Ultra-high-performance Liquid Chromatography Coupled to Hybrid Q-Orbitrap Mass Spectrometry." *ANALYTICA CHIMICA ACTA* 1049 (2019): 141–151. Print.
  Impact factor: 5.123, category: CHEMISTRY, ANALYTICAL, rank: 8/80.
- Marlies E.R. C., M. Udert K, B.A. Arends J, **Huysman S**, Vanhaecke L, McAdam E, Rabaey K. "Membrane Stripping Enables Effective Electrochemical Ammonia Recovery from Urine While Retaining Microorganisms and Micropollutants." *WATER RESEARCH* 150 (2019): 349–357.
  Impact factor: 7.051, category: Water Resources, rank: 1/99.
- De Paepe E, Van Meulebroek L, Rombouts C, **Huysman S**, Verplanken K, Lapauw B, Wauters J, Hemeryck L and Vanhaecke L. "A Validated Multi-matrix Platform for Metabolomic Fingerprinting of Human Urine, Feces and Plasma Using Ultra-high Performance Liquid-chromatography Coupled to Hybrid Orbitrap High-resolution Mass Spectrometry." *ANALYTICA CHIMICA ACTA* 1033 (2018): 108–118.
  Impact factor: 3.927, category: ENGINEERING, CHEMICAL, rank: 22/137.
- **Huysman S**, Van Meulebroek L, Vanryckeghem F, Van Langehove H, Demeestere K and Vanhaecke L. "Development and Validation of an Ultra-high Performance Liquid Chromatographic High Resolution Q-Orbitrap Mass

Spectrometric Method for the Simultaneous Determination of Steroidal Endocrine Disrupting Compounds in Aquatic Matrices." *ANALYTICA CHIMICA ACTA* 984 (2017): 140–150.
Impact factor: 5.123, category: CHEMISTRY, ANALYTICAL, rank: 8/80.

- Folens K*, **Huysman S***, Van Hulle S, Du Laing G. "Chemical and Economic Optimization of the Coagulation-flocculation Process for Silver Removal and Recovery from Industrial Wastewater." *SEPARATION AND PURIFICATION TECHNOLOGY* 179 (2017): 145–151.
Impact factor: 3.927, category: ENGINEERING, CHEMICAL, rank: 22/137.
(* shared first authors)

Proceedings (conferences & symposia)

- **Huysman S**, Vanryckeghem F, Van Langenhove H, Demeestere K, Vanhaecke L. 2016. "Passive Samplers, as Surrogates for Biological Monitoring, to Measure Emerging (micro)pollutants in the Marine Environment." In *Proceedings of the EuroResidue VIII Conference*, 481–485.
- Vanryckeghem F, **Huysman S**, Van Langenhove H, Vanhaecke L, Demeestere K. 2018. "Screening of the Belgian Part of the North Sea Towards Emerging Organic Micropollutants : Comparison of Two SPE-techniques Prior to UHPLC-Orbitrap-HRMS Analysis." In *Communications in Agricultural and Applied Biological Sciences*. Vol. 83.

Abstracts (conferences & symposia)

- **Huysman S**, Van Meulebroek L, Vanryckeghem F, Van Langenhove H, Demeestere K, Vanhaecke L. 2018. "UHPLC-HRMS Based Targeted and Untargeted Screening of Plasticizers in the Marine Environment." In *Communications in Agricultural and Applied Biological Sciences*, 83:36–36.
- Goessens T, **Huysman S,** Goethals P, Dumoulin A, Vanhaecke L, Croubels S. 2018. "Targeted and Untargeted Screening of Multi-class Antimicrobial Drug Residues with High Resolution Mass Spectrometry in Salt and Fresh Water Ecosystems." In *Hormone and Veterinary Drug Residue Analysis, 8th International Symposium, Abstract Book*, 36–36.
- **Huysman S**, Vanryckeghem F, Smedes F, Van Langenhove H, Demeestere K, Vanhaecke L. 2018. "Divinylbenzene Samplers as Surrogate Tool for Biological Monitoring of (micro)pollutants in the Marine Environment." In *Passive Sampling, 10th International Workshop and Symposium, Abstracts*.
- **Huysman S**, Vanryckeghem F, Smedes F, Van Langenhove H, Demeestere K, Vanhaecke L. 2018. "Divinylbenzene Samplers as Surrogate Tool for Biological Monitoring of (micro)pollutants in the Marine Environment." In *Global Food Inegrity, ASSET 2018 Summit, Abstracts*.
- **Huysman S**, Vanryckeghem F, Demeestere K, Vanhaecke L. 2018. "A Metabolomics Approach Towards Identification of Unknown Emerging Pollutants in Marine Waters." In *Benelux Metabolomics Days, Abstracts*.
- Moeris S, Vanryckeghem F, **Huysman S**, Demeestere K, Vanhaecke L, Van Langenhove H, Janssen C, De Schamphelaere K. 2018. "Using No Observed Effects to Identify Main Contributing Micropollutants in Mixture Toxicity Assessment." In *SETAC Europe, 28th Annual Meeting, Abstracts*.
- **Huysman S**, Van Meulebroek L, Vanryckeghem F, Van Langenhove H, Demeestere K, Vanhaecke L. 2018. "Have Plasticizers Already Reached Our

Marine Environment?" In *Book of Abstracts : VLIZ Marine Science Day. Bredene, Belgium, 21 March 2018*, ed. Jan Mees and Jan Seys, 81:15–15. Oostende, Belgium: Vlaams Instituut voor de Zee (VLIZ).

- Vanryckeghem F, **Huysman S**, Van Langenhove H, Vanhaecke L, Demeestere K. 2018. "Ultra-trace Analysis of Pharmaceuticals and Hormones in Marine Waters by Using UHPLC-ORBITRAP-HRMS." In *Hormone and Veterinary Drug Residue Analysis, 8th International Symposium, Abstract Book*, 39–39.
- Moeris S, Vanryckeghem F, **Huysman S**, Demeestere K, Vanhaecke L, Van Langenhove L, Janssen C, De Schamphelaere K. 2018. "Ecotoxicity Testing of Environmentally Realistic Contaminant Mixtures Using Passive Samplers : What Can We Learn from Repeating Toxicity Tests over an Extended Period of Time?" In *Book of Abstracts : VLIZ Marine Science Day. Bredene, Belgium, 21 March 2018*, ed. Jan Mees and Jan Seys, 81:22–22. Oostende, Belgium: Vlaams Instituut voor de Zee (VLIZ).
- Goessens T, **Huysman S**, Goethals P, Dumoulin A, Vanhaecke L, Croubels S. 2018. "Environmental Screening of Multi-class Antimicrobial Drug Residues in Salt and Fresh Water Ecosystems Using Targeted and Untargeted High Resolution Mass Spectrometry." In *Applied Biological Sciences, 23rd National Symposium, Abstracts*.
- Van Acker E, De Rijcke M, **Huysman S**, Vandegehuchte M, Vanhaecke L, De Schamphelaere K, Janssen C. 2018. "Human Exposure to Algal Toxins via Sea Spray Aerosols." In *Book of Abstracts : 53rd European Marine Biology Symposium*, ed. Jan Mees and Jan Seys, 82:155–155. Oostende, Belgium: Vlaams Instituut voor de Zee (VLIZ).
- Vanryckeghem F, **Huysman S**, Van Langenhove H, Vanhaecke L, Demeestere K. 2018. "Targeted Screening by Orbitrap HRMS Reveals the Occurrence of Pharmaceuticals, Personal Care Products and Pesticides in the Belgian Part of the North Sea." In *Book of Abstracts : VLIZ Marine Science Day. Bredene, Belgium, 21 March 2018*, ed. Jan Mees and Jan Seys, 81:121–121. Oostende, Belgium: Vlaams Instituut voor de Zee (VLIZ).
- **Huysman S**, Vanryckeghem F, Van Langenhove H, Demeestere K, Vanhaecke L. 2017. "How Far Have Plasticizers and Additives Penetrated Our Aquatic Environment?" In *SETAC Europe, 27th Annual Meeting, Abstracts*.
- Vanhaecke L, **Huysman S**, Van Meulebroek L. 2017. "The Central Role of Modern Analytical Tools in Studying the Link Between Oceans & Human Health." In *The Ocean and Human Health, VLIZ Science Symposium, Abstracts*.
- **Huysman S**, Vanryckeghem F, Van Meulebroek L, De Paepe E, Van Langenhove H, Demeestere K, Vanhaecke L. 2017. "Targeted, Suspected and Non-target Screening with High Resolution Mass Spectrometry in the Marine Environment : Ready to Go?" In *Belgian Metabolomics Day, Abstracts*.
- **Huysman S**, Vanryckeghem F, Van Langenhove H, Demeestere K, Vanhaecke L. 2017. "Environmental Screening of Organic Micropollutants in Seawater by Coupling a Divinylbenzene Passive Sampling Device and High Resolution Mass Spectrometry." In *The Ocean and Human Health, VLIZ Science Symposium, Abstracts*.

- **Huysman S**, Vanryckeghem F, Van Langenhove H, Demeestere K, Vanhaecke L. 2017. “Environmental Forensics in Seawater Coupling a Divinylbenzene Passive Sampling Device and High Resolution Mass Spectrometry for the Screening of Micropollutants.” In *SETAC Europe, 27th Annual Meeting, Abstracts*.
- De Paepe E, Van Meulebroek L, Rombouts C, **Huysman S**, Verplanken K, Hemeryck L, Wauters J, Lapauw B, Vanhaecke L. 2017. “A Validated Ultra-high Performance Liquid Chromatography Coupled to High Resolution Mass Spectrometry Multi-matrix Polar Metabolomics Fingerprinting Platform.” In *Belgian Metabolomics Day, Abstracts*.
- Vanhaecke L, **Huysman S**, Van Meulebroek L. 2017. “Emerging Contaminants and Toxins in Aquaculture : How Modern Analytical Tools May Aid to Ensure Food and Environmental Safety.” In *SETAC Europe, 27th Annual Meeting, Abstracts*.
- Vanryckeghem F, **Huysman S**, Van Langenhove L, Vanhaecke L, Demeestere K. 2017. “Measuring Emerging Organic Micropollutants in the North Sea Using High-resolution Orbitrap Mass Spectrometry : Method Validation and Occurrence in Harbour and Open Sea.” In *SETAC Europe, 27th Annual Meeting, Abstracts*.
- **Huysman S**, Vanryckeghem F, Demeestere K, Vanhaecke L. 2016. “Is the Divinylbenzene Passive Sampler a Useful Tool for Measuring Hydrophilic Endocrine Disrupting Compounds in the Marine Environment?” In *Passive Sampling, 8th International Workshop and Symposium, Abstracts*, 19–19.
- **Huysman S**, Vanryckeghem F, Van Langenhove H, Demeestere K, Vanhaecke L. 2016. “Is Our North Sea Contaminated with Endocrine Disrupting Compounds?” In *North Sea Open Science Conference 2016 : Abstract Booklet*, ed. Steven Degraer, Vera Van Lancker, Hilde Eggermont, E Balian, D Brosens, S Maebe, N Noé, and P Huybrechts, 73–73. Brussels, Belgium: Royal Belgian Institute of Natural Sciences ; Belgian Biodiversity Platform.

Acknowledgment

Acknowledgment

First of all, I want to start my acknowledgements with thanking you all for attending my PhD-defense, even though the majority of you probably already started reading this section, while I am trying to survive this doctoral defense. For the ones that are trying to pay attention, but got lost somewhere after the introduction: no, I did not spend this PhD trying to save sea turtles. For the ones that are thinking how long this defense will last and that are pretending to be listening, but are actually hungry and are looking forward to the reception: I do not blame any of you, I am as well! So, while I am struggling further into this defense, I would also like to take the opportunity to express my gratitude to all the people that have contributed to my PhD journey.

Almost 4 years ago, I applied for a function as doctoral researcher in a disciplinary BELSPO project. During the interview 4 professors from different disciplines were attacking me with questions. Honestly Prof. Vanhaecke, you were really giving me a hard time (orbitrap technology??). As a consequence (not written with a t ☺) thereof, I was anxious but at the same time also very happy to start my PhD journey at your lab. It only took me a very short time to realize that you were an amazing supervisor, thanks to your enthusiasm, excellent guidance, support, trust and fighting spirit. Furthermore, your review speed is of unseen nature and is unbeatable (which is not completely true; you were beat once by Prof. Christopher Elliott ☺). Therefore, I would like to express my gratitude to Lynn for giving me the opportunity to start a PhD at your lab and for allowing me to grow, not only as a scientific researcher, but also as a person. Additionally, I would like to thank you for improving my yellow marking. I can guarantee you my yellow marker was empty at the end of my PhD! Although Lynn was my SUPERvisor during this PhD journey, I am also very thankful to my co-supervisor Prof. Kristof Demeestere, with his unwavering calmness and never ending curiosity to question everything. Ok, to be honest, I did not always like your questions (which you could obviously not see on my face), but at the end it improved the quality of our research. I really appreciated your positive attitude and your pats on my back.

Additionally, I would like to thank the members of my examination board: Prof. Lutz Ahrens, Prof. Adrian Covaci, Prof. Colin Janssen, Prof. Niek Sanders and Prof. Siska Croubels. They all provided me with insightful comments and feedback which took my PhD dissertation to the next level. Thank you for sharing all your expertise with me!

A special thanks to my closest research-colleague and friend ir. Francis Vanryckeghem. We both started our PhD at the same time, and so we were able to support each other continuously. I couldn't imagine doing this PhD without you as my analytical partner. You were always willing to listen to my whining (when I was hungry or stressed), even when you were tired. Going with you on conferences and sampling campaigns was always fun, which I will never forget! You are an excellent analytical researcher, and I am convinced that you will soon defend your PhD in glance!

I would also like to thank my colleagues at LCA, who went through this adventure with me. First and foremost thanks to Ellen. We had a bit of a difficult start, because I did not get your unique sense of humor (about the organic farmer?), but this all changed in the summer of 2017. That summer, we were the only two "hard" working PhD students at LCA, and so we had no choice but to talk to each other. From then on, I began to appreciate your humor and so we became friends. Going to Victoria Square was a key milestone in our friendship, which was reinforced by our singing talents. So when the time came, it seemed the most logical choice to ask you as Mr. Mittens' aunt. Thank you Ellen for supporting me during the difficult times. I hope we do have many bites and more together in the future. Of course, I would also like to thank my other colleagues. Caroline, you have an amazing way to put things into perspective. When I was upset about the comments by Lynn on my manuscripts, you told me not to worry because the number of comments were still limited relative to the length of my manuscript. Lieven you were always there to support and help me throughout my PhD. You even tried to learn me how to keep my plants from dying. But apparently, I must be a lost cause, as you are taking care of my plant behind my back. Lieselot, thank you for being my aunt at the faculty, our spaghetti lunches were always pleasant. Also a big thanks to Anne, Anneleen, Arno, Bernardo, Eline,

Gabriel, Jella, Julie, Kaat, Kathleen, Margot, Simon, Sophie, Steven and Sven. An extra word of thanks goes to the laboratory technicians, Dirk, Mieke, Joke and Beata. It is sometimes said too little, but thanks for all the advice and assistance. I would also like to thank Soetkin, Hilde, Wendy and Tanja for alleviating the administrative hassle. Similar gratitude to my project and research partners: Prof. Karel De Schamphelaere, Samuel Moeris, Foppe Smedes, Kees Booij, You Gao, Caroline Knockaert, Elien Dewitte, Willy Baeyens, Herman Van Langenhove, Koen Parmentier, Patrick Roose, Argiro-Sylvia Adamopoulou, Katrijn Baetens, Camillie Gaulier, Ruth Lagring, Els Monteyne, Prof. Simon Haughey, Prof. Christopher Elliott, Kilian Smith, Dr. Sarah Stead, Emmanuel Van Acker, Tess Goessens, Mohammad Kazemabad, Marilynn De Graeve , Klaas Schoutteten, Prof. Ann Dumoulin and VLIZ. Finally, I would also like to thank the colleagues of the Laboratory of Foodborne Parasitic Zoonoses and the Laboratory of Microbiology.

Appreciated distractions were provided by friends and family: Albert, Charlotte, David, Emma, Frank, Jacqueline, Kristina, Mathieu, Silke and Tessa for which I am extremely grateful. Not included in this list are my parents, who especially deserve to be acknowledged for their unrelentless support my whole life. Moreover, I thank my parents for supporting my choices and trying to understand my twists. Without the concern of my mother, my garden would have been overgrown by weeds, and trees would have fallen on my house during this 4-year PhD journey.

Last but not least, this whole journey could not have been possible without the help of my soulmate for life. Olivier, thanks for sharing your life with me and giving me all your support. Every day, you make sure I get up in time for work and eat enough vegetables to keep me healthy. Every day, you put a smile on my face, even when it’s hard. Every day, you offer a listening ear and make me the best version of myself! Thanks for always being there and tempering me (which is sometimes necessary). Thank you for everything! I look forward to our future together, especially with Mr. Mittens by our side.

Heusden, May 2019